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IN VITRO EFFECTS OF SOME HOUSEHOLD CHEMICALS ON INFECTIVE LARVAE OF *HAEMONCHUS CONTORTUS* OF GOAT

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ABSTRACT

In vitro effects of some household chemicals on infective larvae (L_3) of *Haemonchus contortus* of goat were screened during the period from January 2002 to April 2003. Solutions of 0.5, 1, 5, 10 and 20 mg / ml were screened for *in vitro* effects on infective larvae (L_3) of *Haemonchus contortus*. The percent non-motile L_3 was increased when immersed in increasing solutions of the household chemicals ($p < 0.01$) except sodium chloride. Of 10 household chemicals, potassium permanganate showed 100% *in vitro* larvicidal effect at 1 mg / ml concentration. Boric acid showed the second highest larvicidal effect (78.95%) followed by alum (71.86%), bleaching powder (71.43%) and borax (68.97%) at their highest concentration of 20 mg / ml.

Key words: Household chemicals, *Haemonchus contortus*, larvae, goats

INTRODUCTION

Parasitism claims to be one of the main obstacles to profitable livestock rearing in Bangladesh. The most important gastrointestinal nematode infection by *Haemonchus contortus* is found to be widely distributed and most pathogenic in goats in Bangladesh. Although little research work has been carried out on *Haemonchus* larval survivability on pasture in relation to climatic factors in Bangladesh, but it can be said that suitable environmental condition and poor hygienic management which favours microclimate of faeces and herbage makes the condition for larval development and survival and thus making Bangladesh a region for its proliferation. Recent research showed that an overall 65.63% goat remains infected with *Haemonchus controutus* in a year in Bangladesh (Shahiduzzaman *et al.*, 2003). Eggs produced by the adult parasites in the abomasum of infected animals are passed in the faeces and then deposited on the pasture.

The present control strategies for ruminant nematodes worldwide are complicated by the problem of anthelmintic resistance, particularly in *Haemonchus contortus* in Australia, New Zealand, South Africa, South America and many other tropical countries. Recently cases of resistance of L_3 larvae have been reported in sheep in Europe (Maingi *et al.*, 1996) and the United States (Uhlinger *et al.*, 1992). Pritchard (1990) and Jackson (1993) have also reported instances of resistance to one or more anthelmintics. Animals that graze are always exposed to parasites and are thus constantly being reinfected; not to mention that routine deworming treatments delay the development of immunity in young animals. In addition, deworming treatments have little effect if the animals are returned to the same larvae infested field. It is therefore important to find out some alternative means other than anthelmintic use to clean the pasture as much as possible to reduce, if not eliminate, the parasites. Possible strategies for this are resting the land, planting, using chemicals to reduce parasite populations, and improving drainage. This is why the present study was designed to develop a repeatable *in vitro* procedure with household chemicals to determine their effects on L_3 motility of *Haemonchus contortus* of goats.

MATERIALS AND METHODS

The study was conducted during the period from January 2002 to April 2003 in the Department of Parasitology, Bangladesh Agricultural University (BAU), Mymensingh.

Collection of parasites

Viscera of goat were collected from local market of BAU. Adult *Haemonchus contortus* were separated using the standard procedure described by Taylor (1934) and Bell (1957). The collected parasites were kept in Petridishes and incubated at 38^0C until required for experimental use. Preliminary morphological studies of these parasites were done in living condition taking representative samples from each collection.

***In vitro* cultivation of L_3**

An *in vitro* culture system has already been established in our laboratory for harvesting the L_3 larvae of gastrointestinal nematodes. Briefly the collected worms were washed for several times with distilled water or phosphate buffer saline (PBS). Then uteri of gravid females were dissected out, crushed gently in a petridish to release eggs. A known volume of PBS was added to eggs and incubated at room temperature (25- 30^{0}C) for 72 hours then transferred to a 100 ml beaker and further incubated until development of L_3 had occurred.

Preparation of solutions

Solutions at different concentration of household chemicals were made on the basis of mg / ml distilled water. Stock solutions of the chemicals were prepared by adding 10 g of chemicals with 100 ml distilled water. From stock solutions 0.5, 1, 5, 10 and 20 mg / ml concentration were prepared. Preliminary studies were conducted to determine the highest concentration of potassium permanganate (PPM) that could be used without lysing the larvae. Solutions greater than 5 mg / ml concentration caused larvae lyses. Dilutions were therefore 0.5 and 1 mg / ml. Dilution of white powdered form chemicals was based on visibility of larvae on solutions to accommodate counting. Solid and crystal formed chemicals were first grinded and filtered to make solutions. Copper sulphate (New life, Bd. Ltd., 26% Cu) and potassium permanganate (coloring agents) were diluted until the solution became transparent at the desired treatment level. All solutions except bleaching powder (calcium hypochlorite) (Rahim Chemical Co. Ltd., 40% Ca) was allowed to mix for 30 minutes with glass stirrer to ensure thorough mixing. Solutions were then filtered with filter paper. The precipitate was discarded and the filtrate used for the remainder of the experiment. Solutions were vortexed immediately and again before use.

Larvae application and measurement

Pre-counted larval stock solution was placed from the beaker into the centrifuge tube to centrifuge at 2000 rpm for 10 minutes. The sediment was then taken as a high concentration of larval source. Briefly larvae were pipetted into the clean and dry 50 x 10 mm glass petridishes from stock larvae solution. A 300 µl aliquot of treatment solution was pipetted into a petridish using a digital micropipette, followed by 100 µl of larvae. Larvae were pipetted from the stock larval solution from approximately the same depth. The petridishes, marked with lines approximately 2 mm apart, were then covered with a lid. The petridishes were then examined under dissecting microscope (70x, Leica Zoom 2000) for counting the number of live and dead larvae.

***In vitro* screening**

Larvae were immersed, in the treatment solutions, in separate trial at a concentration of 1, 5, 10, and 20 mg / ml except in PPM solutions which was used at a concentration of 0.5 and 1 mg / ml. Freshly prepared solutions were used in these trials. Following a 4 hour treatment period at room temperature, non-motile larvae were counted using a dissecting microscope. In order to determine the stagnant L_3 which were motile, observed for 3-5 minutes. If movement resulted, the larvae were considered motile. After all the dishes had been initially counted for non-motile L_3, the total in each dish was counted by adding a drop of lugols iodine to kill all larvae. Percent non-motile L_3 was then calculated for each petridish.

Statistical analyses

Data were subjected to ANOVA for a randomized complete block design (RCBD) with four replicates. Treatment means were further separated using the Duncan's Multiple Rang Test (DMRT). Multiple comparisons among the treatment means for each chemical were tested by using the least significant difference test (LSD).

RESULTS AND DISCUSSION

***In vitro* screening**

The percent non-motile L_3 increased ($p < 0.0001$) when immersed in increasing concentration of household chemicals (Table 1). At each concentration level of the chemicals the percent non-motile L_3 differed ($p < 0.05$), with the exception of 1, 5 and 10 mg / ml solutions of sodium chloride, and 1 and 5 mg / ml solutions of sodium bi carbonate. In case of copper sulphate little changes ($p < 0.018$) occurred in between 1 and 5 mg / ml solutions. Bleaching powder (71.43%) and alum (71.86%) achieved an acceptable degree of effectiveness at their highest concentration. In addition, 20 mg / ml concentration of boric acid was considered quite effective with a 78.95% immobilization.

Table 1. *In vitro* immobilization of L_3 of *Haemonchus contortus* with some household chemicals at different concentrations

Chemicals	Immobilization (%)					SE[a]	LSD[b]
	0.5 mg/ml	1 mg/ml	5 mg/ml	10 mg/ml	20 mg/ml		
Bleaching powder	–	0	08.30	14.28	71.43	1.16	1.38
Borax	–	0	09.09	44.44	68.97	0.80	1.85
Boric acid	–	0	31.25	59.09	78.95	1.55	3.12
Calcium carbonate	–	0	17.46	30.00	56.79	1.13	2.38
Copper sulfate	–	11.11	13.79	44.44	60.00	1.04	2.22
Alum (Fitkiri)	–	0	11.76	63.64	71.86	1.60	4.59
Sodium carbonate	–	0	08.82	47.06	53.13	0.94	2.19
Sodium chloride	–	0	0	0	41.18	0.33	0.75
Sodium bicarbonate	–	0	0	33.33	44.44	1.21	2.44
Potassium permanganate	60.5	100	–	–	–	3.45	7.25

[a]Standard error derived from statistical model, [b]Least significant difference at $p < 0.05$, – = Not done.

Calcium carbonate, sodium carbonate and copper sulphate were considered less effective with percent non-motile L_3 of 56.79, 53.13 and 60 respectively whereas sodium chloride and sodium bicarbonate were considered ineffective with 41.18% and 44.44% immobilization respectively at 20 mg / ml concentration. In case of bleaching powder and sodium chloride the marked changes occurred in immobilization between 10 and 20 mg / ml treatment levels. Alum was effective at 10 mg / ml with 63.64% non-motile L_3 whereas borax was 68.97% at 20 mg / ml treatment level. *In vitro* larvicidal effects of PPM resulting in a 100% immobilization at the concentration of 1 mg / ml (Table 1). The potent larvicidal effects ($p < 0.0001$) was found with highest concentration (20 mg / ml) of bleaching powder, borax, boric acid and alum. The results indicated that boric acid was more effective than other compounds.

The chief reaction of bleaching powder and of hypochlorites in general, is due to the hypochlorite ion, which possesses active oxidizing properties. Many researchers have documented sodium hypochlorite (household bleach) as an effective exsheathing agent for nematodes (Conder and Johnson, 1996), even when used at concentration lower than 1% (Glaser and Stoll, 1940; Campbell and Gaugler, 1991). The result is in conformity with that of bleaching powder resulting in a more or less similar action on immobilization of infective larvae.

Potassium permanganate is a powerful oxidizing agent and it provides its oxidation properties in acidic, neutral and alkaline medium. Therefore its use in soils of different pH facilitate to exert its potent larvicidal actions. In this study, PPM used at concentration of 1 mg / ml effectively immobilized larvae (100%).

Borax and boric acid are the extensive sources of boron. The requirement of boron for fertilizing the soil ranges from 0.2 to 0.5 mg / ml (Thompson and Troeh, 1987). Very little work has been done for larvicidal action of boric acid previously but from this study it can be said that boric acid has potential larvicidal action at a concentration of 20 mg / ml. The poor response of calcium carbonate observed suggests that it may lose its abrasive and desiccating qualities when put in water solution. Munir *et al.* (2001) found that calcium carbonate, also called chalk, in 10% concentration had profound negative effect on egg hatch to L_1, indicating its destructive effect on egg and larvae of *Haemonchus contortus*.

The present study showed that the copper sulfate at a concentration of 20 mg / ml caused 60% immobilization of L_3 while Paciejewski (1994) observed no death of infective larvae in 1:1000 and 1:2000 concentrations. This contrast in relation to larvicidal activity of copper sulphate might be simply due to the low level of concentration used by Paciejewski (1994). Aluminum sulphate, also called alum showed considerable degree of effectiveness. So, its use to forage crops and sewage can be effective to kill L_3. Alum is water soluble and possesses remarkable power of precipitating colloids. The larvicidal effects of sodium carbonate and sodium bicarbonate recorded in this study were not effective as like as others. Munir *et al.* (2001) observed that there was a gradual decrease in hatching of eggs of *Haemonchus contortus* with increasing concentration of sodium choride. Sodium has effect on swimming speed of *Schistosoma mansoni* (Elizabeth, 1982). According to Nunnery (1953) sodium chloride is appropriate for use against

Ancylostoma larvae. Munir *et al.* (2001) investigated that sodium chloride had effect on hatching of *Haemonchus contortus* eggs to L_1, L_2 and L_3. Only poor response observed at 20 mg / ml solutions in this trial may not be considered ineffective until and unless further trials at concentration higher than 20 mg / ml are conducted.

The results of the *in vitro* experiments presented here indicate that potassium permanganate, boric acid, alum and bleaching powder among the household chemicals are highly effective larvicides. Further studies in pastures with these chemicals are required to use them strategically to lessen worm burdens during periods when parasites are naturally more abundant, such as in late spring, early summer, or when warm and wet weather conditions prevail. Spraying the barns, barnyards, livestock premises and sewage where excreta accumulate, with safer doses of these chemicals may help to prevent or reduce nematode contamination. Larvicidal properties of the household chemicals could decrease gastrointestinal parasitic load, may reduce producers dependency on traditional anthelmintics to control infective larvae as well as the potential anthelmintic resistance when used strategically with pasture fertilization and other agricultural practices. Additional field studies are needed to corroborate results presented herein.

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