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

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ABSTRACT
In vitro effects of some household chemicals on infective larvae (L3) of Hamarchus contortus of goat were screened during the period from January 2002 to April 2003. Solutions of 0.1, 1, 5, 10 and 20 mg/ml were screened for in vitro effects on infective larvae (L3) of Hamarchus contortus. The present non-metate L3 was screened when immersed in increasing solutions of the household chemicals (p<0.01) except sodium chloride. Of 10 household chemicals, pramoxine hydrochloride showed 100% in vitro larvicidal effect at 1 mg/ml concentration. Boric acid showed the second highest larvicidal effect (78.95%) followed by sodium (75.6%), quinine hydrochloride (71.49%) and benz (69.97%) at their highest concentration of 20 mg/ml.

Key words: Household chemicals, Hamarchus contortus, larvae, goats

INTRODUCTION
Parasitism is one of the main obstacles to profitable livestock rearing in Bangladesh. The most important gastrointestinal nematode infection by Hamarchus contortus is found to be widely distributed and most pathogenic in goats in Bangladesh. Although little research work has been carried out on Hamarchus larval survivability on pasture in relation to climatic factors in Bangladesh, but it can be said that suitable environmental condition and poor hygiene management which favours microclimate of faeces and herbage make the condition for larval development and survival and thus making Bangladesh a region for its proliferation. Recent research showed that an overall 55.63% gox remains infected with Hamarchus contortus 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 resistant nematodes worldwide are complicated by the problem of anthelmintic resistance, particularly in Hamarchus contortus in Australia, New Zealand, South Africa, South America and many other tropical countries. Recently cases of resistance of L3 larvae have been reported in sheep in Europe (Mangold et al., 1996) and the United States (Uhlinger et al., 1992). Pyrvinium (1990) and Jackson (1993) have also reported instances of resistance to ivermectin and others. Anthelmintic drugs that 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 pasture laden field. It is therefore important to find out some alternative strategies other than anthelmintic use to clean the pasture as much as possible to reduce, if not eliminate, the parasite. Possible strategies for this are resting the plant, 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 L3 viability of Hamarchus 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 Hamarchus contortus were separated using the standard procedure described by Taylor (1934) and Bell (1957). The collected parasites were kept in Petri dishes and incubated at 26°C until required for experimental use. Preliminary morphological studies of these parasites were done in vivo condition taking representative samples from each collection.

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In vitro cultivation of L.3

As in vitro culture system has already been established in our laboratory for harvesting the L.3 larve of gasterinostelial nematodes. Briefly the collected worms were washed for several times with distilled water or phosphate buffer saline (PBS). Then uterus of gravid females were dissected out, crushed gently in a petri dish to release eggs. A known volume of PBS was added to eggs and incubated at room temperature (25-30°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 lysis. 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 dialuted until the solution became transparent at the desired treatment level. All solutions except bleachng powder (calcium hypochlorite) (Rahim Chemical Co. Ltd., 40% Ca) was allowed to mix for 10 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 centrifugate 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 larval solution. A 500 µl aliquot of treatment solution was pipetted into a petri dish using a digital micropipette, followed by 100 µl of larval. 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 Range 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 bicarbonate. In case of copper sulphate little changes (p < 0.018) occurred in between 1 and 5 mg / ml solutions. Bleaching powder (71.45%) 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 L0 of *Haemoncus contortus* with some household chemicals at different concentrations.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Immobilization (%)</th>
<th>SE1</th>
<th>LSD2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 mg/ml 1 ml</td>
<td>5 mg/ml</td>
<td>10 mg/ml</td>
</tr>
<tr>
<td>Bleaching powder</td>
<td>0</td>
<td>08.30</td>
<td>14.28</td>
</tr>
<tr>
<td>Borax</td>
<td>0</td>
<td>09.09</td>
<td>44.44</td>
</tr>
<tr>
<td>Boric acid</td>
<td>0</td>
<td>31.25</td>
<td>59.30</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0</td>
<td>17.46</td>
<td>50.00</td>
</tr>
<tr>
<td>Copper sulphate</td>
<td>0</td>
<td>11.11</td>
<td>13.79</td>
</tr>
<tr>
<td>Alum (Potassium)</td>
<td>0</td>
<td>11.76</td>
<td>63.64</td>
</tr>
<tr>
<td>Sodium carbonate</td>
<td>0</td>
<td>08.82</td>
<td>47.06</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0</td>
<td>00.00</td>
<td>41.18</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>0</td>
<td>33.33</td>
<td>44.44</td>
</tr>
<tr>
<td>Potassium permanganate</td>
<td>60.5</td>
<td>100</td>
<td>-</td>
</tr>
</tbody>
</table>

1Standard error derived from statistical model. *p* < 0.05:
2Not done.

Calcium carbonate, sodium carbonate and copper sulphate were considered less effective with pent-non-mono L0 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-mono L0 whereas borax was 68.97% at 20 mg/ml treatment level. In *in vitro* larvalidal effects of PPM resulting in a 100% immobilization at the concentration of 1 mg/ml (Table 1). The potent larvalidal effects (p < 0.0001) was found with highest concentration (20 mg/ml) of bleaching powder, borax, boric acid and alum. The results indicated that borax and boronic acid were 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 extirpating agent for nematodes (Conder and Johnson, 1996), even when used at a concentration lower than 1% (Glaser and Stoll, 1946; 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 extirpate its potent larvicidal actions. In this study, PPM used at concentration of 1 mg/ml effectively immobilized larva (100%).

Borax and boric acid are the extensive sources of boron. The requirement of boron for fertilising the soil ranges from 0.2 to 0.5 mg/ml (Thompson and Truitt, 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. Muni et al. (2001) found that calcium carbonate, also called chalk, in 10% concentration had produced negative effect on egg hatch to L0, indicating its destructive effect on egg and larvae of *Haemoncus contortus*.

The present study showed that the copper sulphate at a concentration of 23 mg/ml caused 60% immobilization of L0 while Pacejewski (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 Pacejewski (1994). Aluminium sulphate, also called alum showed considerable degree of effectiveness. So, its use to forage crops and sewage can be effective to kill L0. 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. Muni et al. (2001) observed that there was a gradual decrease in hatching of eggs of *Haemoncus contortus* with increasing concentration of sodium chloride. Sodium has effect on swimming speed of *Schistosoma mansoni* (Elizabeth, 1982). According to Nummer (1953) sodium chloride is appropriate for use against...
Acynetaon larv. Murer et al. (2001) investigated that sodium chloride had effect on hatching of Haemonchus contortus eggs to L1, L2, and L3. Only poor response observed at 20 mg/l solutions in this trial may not be considered ineffective until and unless further trials at concentration higher than 20 mg/l 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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REFERENCES