THE CYST WALL OF *UNICAUDA LAUMAE* RAHEMO (MYXOSPORIDIA: MYXOBOLIDAE) FROM THE LIVER OF *BARBUS GRYPUS* HECKEL

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SOME information is available on parasites of fishes and the diseases caused by them[1–8]. Two species of Microsporidia, *Nosema ovoideum* and *Octospora machari* have been reported[9] to cause serious liver damage. They cause liquified ulcer.

Of the 15 species of *Unicauda*, only *U. laumae* has been reported[10] to infect liver tissue. The present work is a study of the histochemical nature of the cyst wall of *U. laumae* in the liver of the teleost, *Barbus grypus*.

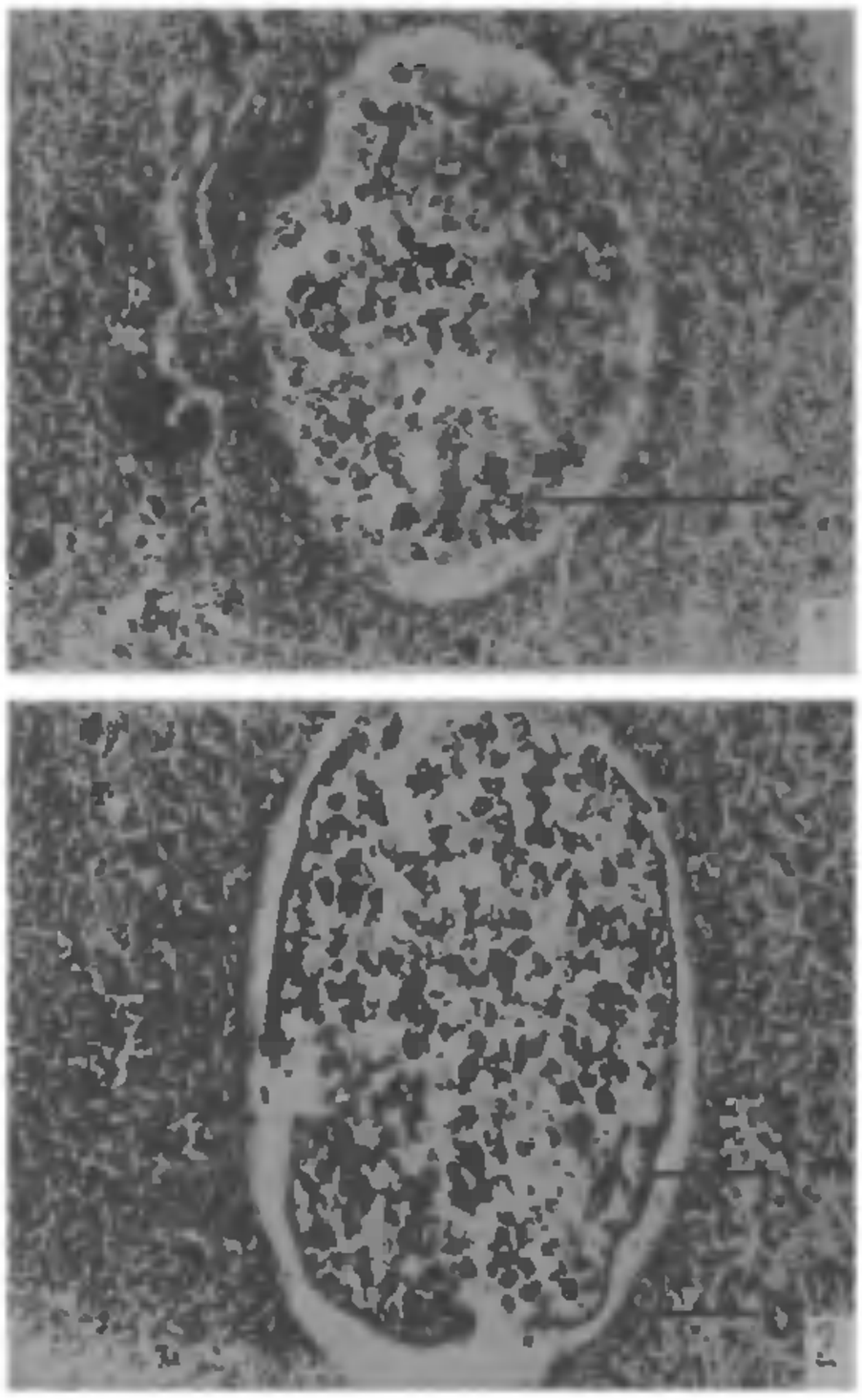
For microscopic examination, pieces of infected and uninfected liver were fixed in Bouni's fluid, embedded in wax, sectioned into 8 μm and stained with Delafield haematoxylin-eosin. For histochemical study, pieces were fixed in Zenker fluid and 10% neutral formalin, embedded in wax, sectioned into 8 μm and treated with the following histochemical tests[11]:

1. Periodic acid-Schiff (PAS) technique after McManus.
2. Diastase digestion test (30 min at 32°C) followed by PAS reaction.
3. Alcian blue (AB) technique after Steedman.

Cysts of *U. laumae* are clearly seen with the naked eye. Some of these cysts are on the surface of the liver; others are quite deep. The liver is often congested due to high infection.

Examination of haematoxylin-eosin stained preparations revealed that sporozoids which invade the liver cause disintegration of the tissue, form cysts, develop cyst wall and subsequently become almost isolated from the surrounding tissue (figure 1). The cyst is surrounded by condensed liver cells due to the pressure caused by the huge increase in the number of spores by sporogonic division. The liver tissue surrounding the cyst has a conspicuous increase of red blood corpuscles, probably due to hyperaemia[12]. Other histopathological changes such as xenoma or granuloma, like those caused by Microsporidia[2], could not be observed.

It appears that the cysts are double-walled. The outer wall is thicker than the inner one (figure 2).



Figures 1 and 2 (×340) **1.** Horizontal section through the liver of *Barbus grypus* showing spores (S). Bouin, haematoxylin-eosin; **2.** Horizontal section through the liver of *Barbus grypus* showing the outer (O) and inner (I) cyst walls. Zenker, PAS.

The outer wall is cellular and is of the host origin, whereas the inner one shows acellular nature and possibly is of parasite origin.

The spores of *Unicauda laumae* showed different staining affinities to eosin. Some are eosinophilic while others are almost unstained. This may be attributed to their different stages of maturation[5]. Histochemical tests revealed that the spore has two walls, an outer thick wall which stains bright purplish-red with both PAS and diastase test, whereas the inner wall stains dull purplish-red with PAS and very faint purple with diastase test (figure 2). The outer wall is negative to alcian blue test while the inner one shows weak positivity. Thus, the two walls are histochemically distinguishable. The outer cellular wall is composed of complex carbohydrates, glycolipids and glycolipoproteins while the inner acellular wall is composed of a moiety of complex carbohydrates and acid mucopolysaccharides.

11 November 1986; Revised 17 February 1987

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INDIGENOUS PLANT OILS AS LARVICIDAL AGENT AGAINST *ANOPHELES STEPHENSI* MOSQUITOES

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Mosquitoes serve as vectors of several diseases causing serious health problems to human beings. Although eradication of these vectors was considered possible by the use of chemical insecticides, development of insecticide resistance initiated a search for alternative control measures[1]. Biologically active plant extracts are therefore studied for their efficacy to kill larvae of different mosquitoes[2–6]. Supavarn *et al*[2] reported that of the 36 plant samples studied, five of them killed all the larvae of *Aedes aegypti* within 7 days at a concentration of 1000 ppm. Joshi *et al*[3] found natural and synthetic garlic to be an effective larvicide. The present study was carried out to determine the larvicidal activity of oils from 10 plants of 8 genera viz *Cedrus deodara*, *Cymbopogon nardus*, *C. flexuosus*, *C. martini*, *Lavandula officinalis*, *Mentha arvensis*, *Ricinus communis*, *Eucalyptus globulus*, *Eugenia caryophyllus* and *Melia azadirachta* against laboratory colonized fourth instar larvae of malaria vector *Anopheles stephensi*.

The larvae of *A. stepheni*, used in this study were reared in CDRI insectary at a temperature of 26±2°C and a relative humidity of 75±5%. The fourth instar larvae were taken to study the larvicidal activity of different plant oils.

Different parts of the plants were cut into pieces and oil was obtained by steam distillation and then purified to get rectified oil. It was diluted thrice in acetone and added to 150 ml of dechlorinated tapwater to obtain the desired concentration.

All bioassays were performed according to the standard method[7]. Fifty larvae were tested at each dilution—25 larvae in one crystallizing dish (Borosil, 100×50 mm) in 150 ml of dechlorinated tapwater. All oils were tested at 9 different dilutions viz 25, 50, 75, 100, 125, 150, 175, 200 and 250 ppm. A small aliquot of yeast powder was supplied for nutrition. With each experiment, a set of control was included with 5 ml acetone. Larvae mortality was recorded after 24 hr. Toxic activity was reported as LC_{50}, that is ppm of oil that killed 50% larvae in 24 hr.

The results of larvicidal activity of different plant oils against fourth instar larvae of *A. stephensi* are presented in table 1. The maximum activity was observed in the case of *C. deodara* which caused 50% mortality at dose of 63.2 ppm. To our knowledge no reports are available regarding insecticidal activity of *C. deodara* except one report which showed it to be toxic for adult *A. stephensi* mosquitoes[8]. Oils from *C. nardus*, *C. flexuosus*, *C. martini*, *L. officinalis*, *M. arvensis*, *R. communis*, *E. globulus*, *E. caryophyllus* and *M. azadirachta* showed LC_{50} values of 105.4, 91.4, 100.0, 83.6, 83.8, 113.0, 98.5, 96.5 and 88.5 ppm, respectively. Earlier reports[2,5] showed a need for very high concentration of plant extracts for achieving significant mortality of mosquitoes larvae, while oils used in the present study resulted in 100% mortality at dose of 250 ppm in all the cases. The most effective *C. deodara* oil caused complete mortality even at a concentration of 175 ppm.

The present study indicates the efficacy of several plant oils as larvicidal agents and their possible use in the biological control of *A. stephensi*, an important vector for several tropical parasitic diseases. Combined with conventional chemical larvicides