INHIBITION OF CALCIUM DEPOSITION BY CHEMICAL INHIBITORS

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FREE swimming larvae of the marine organisms like barnacles, bivalves or tubeworms become a fouling menace only when they settle and secrete their calcareous shells. Crisp and Austin[1] have presented adequate experimental data to suggest that toxic copper pigment contained in antifouling compositions prevents barnacle growth by affecting adversely the latter's calcium deposition ability. Wilbur[2] and others have observed that chemical compounds like 2. 4-dinitrophenol, sulphanilamide and sodium fluoride inhibit calcium deposition rates in oyster species. Added to these observations, Karande[3] noted that one structurally very poor timber, *viz.*, *Tetrameles nudiflora* survived *Teredid* attack over a period of four years in *Teredo* infested waters. A critical examination of the veligers which were also induced to attack this timber under controlled laboratory conditions confirmed that their inability to metamorphose was due to the failure of calcium deposition activity. The chemical constituents of the timber, like alkaloids[4], presumably interfered with the shell deposition ability of the veligers.

With a view to examining this problem of inhibition of calcium secretion by the chemical inhibitors in shell dwelling molluscs and crustaceans, a laboratory screening of nine different chemical compounds using veliger larvae of *Teredo furcifera* as the test-organism was carried out (Fig. 1). This note gives salient features of the screening procedure and the results obtained.

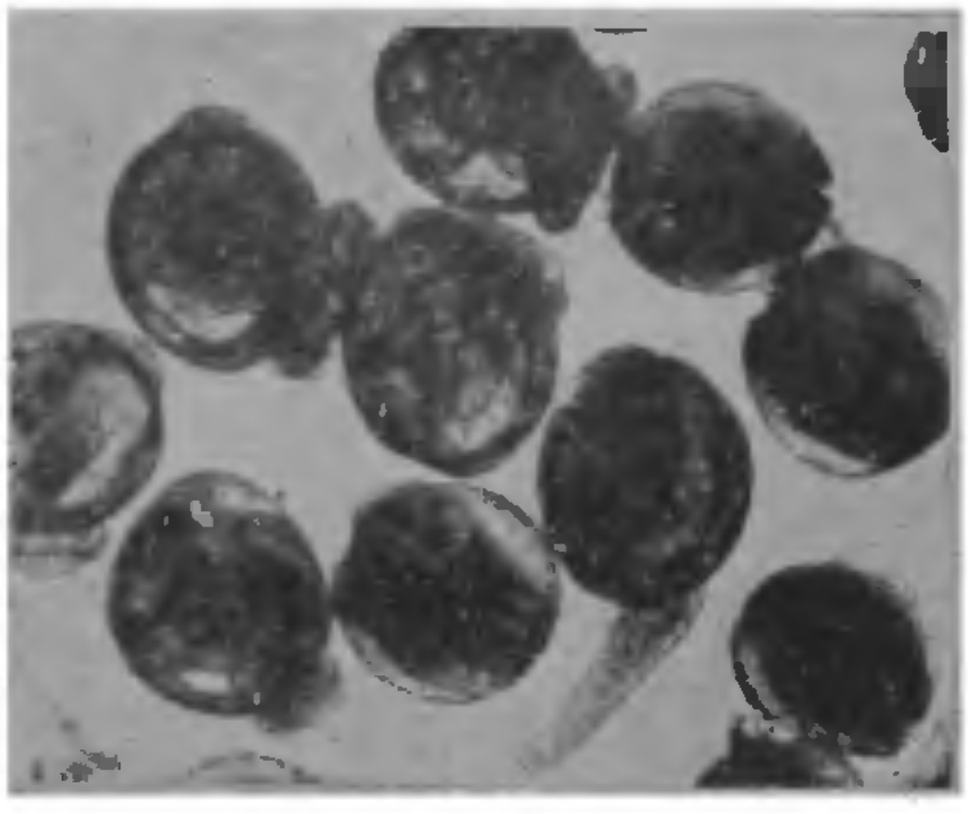

FIG. 1. Veliger larvae of *Teredo furcifera* having chitious bivalve shells, actual size 285 μ.

In veliger larvae four growth stages with reference to shell formation and the burrow lining are recognised. These are stage 1, pediveliger larvae having teeth on gaping margins of the chitinous shell; stage 2, beginning of calcification of the gaping margins; stage 3, secretion of the mucous material around the entry hole and its subsequent impregnation with calcium carbonate and stage 4, formation of calcium carbonate shield over the burrow and the development of a partition dividing the burrow into two halves, one each for the excurrent and the incurrent siphons (Fig. 2).

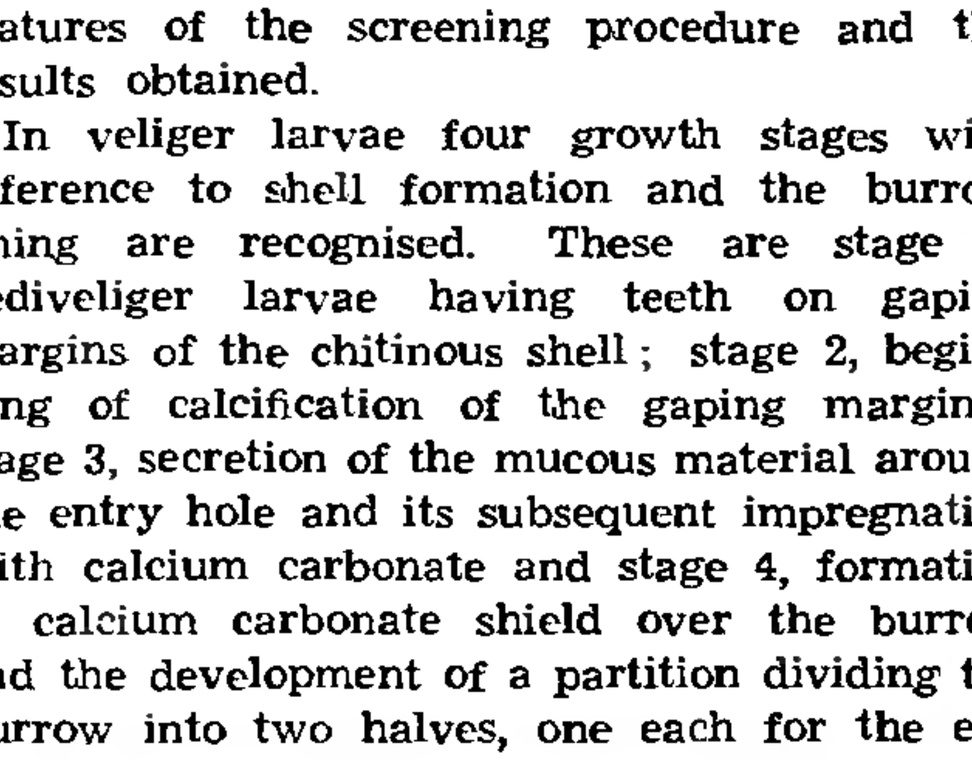

FIG. 2. Showing calcium carbonate shields guarding the entry-holes of young *Teredo furcifera*, 4th stage, actual size 350 μ approximately.

Generally 80–85 p.c. of the veligers successfully attack the timber and achieve 4th stage at the end of 4 days growth. The worms need no extraneous food for the sustenance and may live for a considerable period on a timber they burrow and ingest[5].

Wooden test pieces of *Abies pindrow* harbouring hundred or more stage 1 pediveligers were exposed for two days to the graded solutions of chemical compounds in 500 ml capacity crystalline jars. Karande *et al.*[6] have earlier examined the behaviour of the larvae under varying experimental conditions. Table II gives the concentration of each chemical compound and its presumed action on the shell generation. The control test-pieces were

simultaneously maintained in inhibitor-free sea-water. Test-pieces were also exposed to two more chemical compounds reported as having beneficial effect on the shell building process amongst bivalve molluscs. The degree of inhibitory action of a compound was judged by its ability to prevent or retard the inpregnation rate of calcium carbonate in the mucous film lining the entry holes of the veligers. A calcium deposition capacity of the test organisms, as judged by the frequency of occurrence of growth stages, is found to have retarded with the increasing concentration of each of the compounds tested. Table I gives typical results of one of the compounds examined, *viz.*, 2, 4-dinitrophenol. At the lowest concentration of this compound as many as 67 p.c. of the larvae undergo complete transformation and achieve fourth stage of growth whereas at the higher concentrations only about 5 or 6 p.c. of them achieve 4th stage.

TABLE I

Showing inhibitory effect of 2, 4-*dinitrophenol on shell growth of* Teredo furcifera

2, 4-Dinitrophenol concentration	Per cent occurrence of growth stages of veligers			
Dilution- (p.c.) (·000125 M)	Stage 1	Stage 2	Stage 3	Stage 4
25	29	0	4	67
50	48	0	6	46
75	82	5	7	6
100	84	5	6	5

Table II gives per cent success of the shell formation at four varying concentrations of nine different chemical compounds examined. In respect of first seven compounds with the increasing concentration, there has been a gradual fall in the shell deposition activity of the veligers. In respect of last two compounds which are expected to act as accelerators, the shell development is generally higher at all the four concentrations, if not better than in controls.

Interestingly, present results closely agree with those reported by other workers, where method[2] other than ours was adopted for the screening. In almost all the chemical compounds screened by us, the test-organisms survived as long as 15 days, that being a normal span of life of a veliger, but failed to deposit adequate $CaCO_3$ which is so vital for their metamorphosis and growth.

The investigation under report has brought out that the shell growth of the marine organisms is inhibited by certain chemical compounds even at very low concentrations. The bioassay method employed here may prove of advantage in screening the natural and synthetic chemical compounds for their antifouling efficacy. The method can also be used for the analysis of water samples from oyster or shrimp farms or from the other polluted areas in the harbour.

TABLE II

Inhibitory effect of various chemical compounds on calcium deposition in Teredo furcifera

Chemical compound	Presumed action	Reported rating (Wilbur, 1964) per cent of normal deposition in oysters	Present rating, per cent success of shell deposition at 4-graded concentrations			
			25%	50%	75%	100%
Monoiodoacetate (·001 M) ..	Reacts with SH groups	19·6	0	0	0	0
Sodium fluoride (·01 M) ..	Inhibits glycolysis	45·2	40	43	18	3
2, 4-Dinitrophenol (·000125 M)	Reduces high energy phosphate concentration	10·8	67	46	6	5
Toluene-*p*-sulphonamide (50 mg/litre)	Inhibits carbonic-anhydrase	63	48	0	3	2
Benzene sulphonamide (50 mg/litre)	,,	63	83	50	27	46
Sulphanilamide (50 mg/litre) ..	,,	68	8	4	14	13
Beryllium nitrate (·0.01 M) ..	Inhibits alkaline phosphatase	13·9	35	5	6	13
Sodium succinate (·01 M) ..	Respiratory substrate	110	65	58	76	52
Sodium maleate (·01 M) ..	,,	120	74	65	32	57

The authors wish to thank Shri C. P. De, Director, N.C.M.L., Bombay, for his interest in this investigation.

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