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In Vitro TESTING OF STERILANTS FOR THE DISINFECTION OF THE EGG MASSES OF ROOT-KNOT NEMATODE (*Meloidogyne incognita*)[1/]

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Under gnotobiotic conditions, it is necessary that the nematodes should be axenized before studying the plant-nematodes interactions (*3, 9*). Axenization involves only surface sterilization. A review of methods for the sterilization has been given (*2, 9*). However, a quick and precise method for the disinfection of the egg masses of root-knot nematode *Meloidogyne incognita* was still lacking. The problem of checking nematode sterility as well as that of soil, plants and other components of a gnotobiotic system, has not received sufficient attention. A few workers (*1, 9*) have considered it important to test for fungal contaminants and bacteria under sterile tissue culture conditions. This system of plant biotechnology offers a number of advantages: (i) transferring alien genes for disease and nematode resistance; (ii) amplification of genetic variation from variants which shall be screened for disease and pest resistance, at the cellular level; (iii) investigations in isolation; (iv) effect of various biotic and abiotic factors in a test tube; (v) quick screening of nematicides; (vi) screening of crop germplasm; (vii) studying feeding behaviour of phytonematodes (*4, 7*).

In the present investigation seeds of okra were surface sterilized with 0.4% mercuric-chloride solution for 12-15 minutes. Seeds were then rinsed three times with sterile distilled water, transferred to test tubes containing plain agar medium and placed in the dark for germination. Root-tips (1-2 cm long) excised from 3-5 day-old aseptically grown seedlings of okra (*Abelmoschus esculentus* cv. Pusa Sawani) were cultured on Murashige and Skoog's medium (MS-1962) supplemented with IAA (0.1 mg/l) + Kinetin (0.1 mg/l) and on plain agar media (*4,5*).

The excised root-segments, thus raised were infected with surface-sterilized egg masses of *M. incognita* (2 egg masses per test tube; one egg mass containing approx. 400 eggs) with various organic and inorganic sterilants at various concentrations viz. mercuric chloride (0.1, 0.01 and 0.001%), aretan (0.1, 0.01 and 0.001%), sodium hypochlorite (2, 5 and 7%) and coppersulphate (0.1, 0.01 and

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TABLE 1 - Per cent disinfection and root penetration of root-knot nematode (M. incognita) treated with different organic and inorganic disinfectants in excised roots of okra on two media (MS = Murashige & Skoog, PA = Plain agar)

Treatment	Conc. (%)	Mercuric chloride			Aretan			Sodium hypochlorite			Copper sulphate		
		0.1	0.01	0.001	0.1	0.01	0.001	2	5	7	0.1	0.01	0.001
		Per cent sterile cultures											
Disinfection	MS	80*	60	20	40	60	40	60	80	60	20	-	-
	PA	90	80	60	70	80	60	80	90	80	60	50	40
Root Penetration	MS	46** ±5.1	44 ±4.9	42 ±3.2	41 ±3.0	45 ±5.0	44 ±4.1	45 ±4.2	45 ±5.1	39 ±3.5	43 ±4.9	41 ±4.3	40 ±3.2
	PA	25 ±6.1	20 ±5.0	15 ±4.6	10 ±2.3	20 ±5.9	5 ±2.9	10 ±3.4	15 ±2.2	10 ±2.0	10 ±2.3	5 ±3.1	5 ±2.0

* Average of 10 repeats in percentage

** Mean number of juveniles that penetrated ± S.E.

0.001%), respectively. Each treatment had 10 repeats. The time for sterilization of the egg masses was kept as 15 minutes. The root cultures were incubated in the culture room at 25± 2ºC, which was well illuminated (approx. 5000Lx). A Laminar Flow Chamber (Klenzaids, Bombay) was used for all experimentation under sterile conditions. Root penetration by the juveniles was observed 3 days after inoculation. The data was statistically analysed.

A glance at Table 1 shows a differential response for disinfection in two media with the sterilant as well as its concentration. Percent sterile cultures found disinfected were maximum (80% in MS=Murashige and Skoog and 90% in PA= Plain Agar) in mercuric chloride (0.1%) and sodium hypochlorite (5%). Copper sulphate was least effective at 0.1%. The juveniles started to hatch from the egg masses within a day in the excised root cultures, and the larvae moved towards the root tips and searched for a penetration site. However, the root penetration by the juveniles was comparatively high in the okra, being a favourite host of *M. incognita* when the egg masses were sterilized with 0.1% H_gCl_2.

To conclude, egg masses sterilization and the root penetration studies of the root-knot nematode under sterile conditions in tissue cultures emphasize its utility as an effective tool not only for studying the life-cycle in isolation in root-cultures (*6,8*) but also for the evaluation of various nematicides and organic and inorganic sterilants under strictly controlled environment (*7*). There is also the possibility of using this technique for selecting nematode-resistant germplasm cell lines from the nematode infected root callus cultures (*8,9*).

RESUMO

(TESTE «IN VITRO» DE ESTERILIZANTES PARA DESINFECÇÃO DE MASSAS DE OVOS DO NEMATÓIDE DAS GALHAS (*Meloidogyne incognita*))

Sementes de quiabo, previamente desinfetadas com bicloreto de mercúrio, foram colocadas para germinar em tubos com ágar-água. Após germinação e desenvolvimento, pontas de raízes foram cortadas e colocadas em tubos com o meio nutritivo de Murashigi e Skoog. Com o desenvolvimento das raízes, duas massas de ovos de *Meloidogyne incognita* foram colocadas em cada tubo, após desinfecção com quatro produtos químicos, em diferentes concentrações, durante 15 minutos. Os dois melhores tratamentos foram: bicloreto de mercúrio a 0,1% e hipoclorito de sódio a 5%. Aretan e sulfato de cobre, nas concentrações de 0,1, 0,01 e 0,001%, foram menos eficientes. Nenhum dos tratamentos afetou a taxa de penetração nas raízes das larvas que eclodiram dos ovos tratados.

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