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SHORT COMMUNICATIONS

CULTURING OF BURROWING NEMATODE, *RADOPHOLUS SIMILIS* ON CARROT DISCS*

BY

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Conduct of pathogenicity experiments are dependant on the availability of large sterile nematode populations. Earlier attempts in this direction were initiated with the development of excised root culture with *Pratylenchus neglectus* as the test organism (Mountain, 1955). O'Bannon & Taylor (1968) employed the technique of carrot discs for obtaining large population of *Pratylenchus brachyurus* and *Radopholus similis*. In our pathogenicity experiments with *Radopholus similis* on coconut, we encountered difficulties in raising large populations of the burrowing nematode because of the high percentage of tannin in coconut roots and the resultant effects on nematode population itself as well as the sieves (Koshy *et al.*, 1979). The massive root system and the perennial nature of coconut palms, make it imperative that unless huge populations are available, pathogenicity experiments may not be

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fruitful. An attempt was, therefore, made to develop a technique for raising large sterile populations of the burrowing nematode under laboratory conditions. The technique developed and reported here is essentially based on the method of O'Bannon and Taylor (1968) with certain modifications to improve upon the efficiency of raising the culture.

Fresh carrot tubers (*Daucus carota* L.) variety 'Lono', were thoroughly washed in water and dipped in 95% ethyl alcohol. These were then flamed, pared and sliced into discs of 8-10 mm thickness. One such disc was placed on 1% water agar (40 ml) contained in 100 ml Erlenmeyer conical flasks. These flasks were left in a covered enclosure in the laboratory for 48-72 hours to observe for contaminations, if any, and also for an initiation of callus growth. The nematodes extracted from coconut roots were poured through a double layered face tissue paper placed on an aluminium wire mesh support followed by placement of the wire mesh on a petri dish containing sterile water. The extracted nematodes were pipetted into sterile centrifuge tubes and run at 3000 rpm for one minute. The supernatant was decanted leaving about 0.5 ml suspension at the bottom of the tubes. Mercuric chloride (0.1%) was then added to the tubes and the centrifuge was run again for one minute followed by removal of the supernatant and rinsing of the nematode suspension twice with sterile water, with 15 seconds centrifugation each time. The mercuric chloride treated population was then washed thoroughly with 0.1% streptomycin sulphate, similar to mercuric chloride. About 0.5 ml of this treated population was then drawn out with a sterile syringe (needle No. 20) and released on or to the sides of carrot discs in the conical flasks placed on a laminar flow bench. The inoculated flasks were then stored in dark at 20-25°C.

To avoid constant handling and frequent centrifugation of nematodes at various stages, a method was evolved and used subsequently. A glass tube of 10 mm internal diameter and 10 cm length was taken. One end of the tube was closed with a small disc (fixed with araldite) of 13 mm diameter 400 mesh (38 μ stainless steel net). A plastic ring of 2 mm thickness (10 mm inner diameter) was fixed on to the net from below with araldite for giving support to the screen which was suitably trimmed later. The nematode suspension was poured into the tube and then dipped in 5 ml 0.1% mercuric chloride contained in a 10 ml sterile beaker for one minute followed by two washings in sterile water. The same process was repeated with 0.1% streptomycin sulphate. The treated nematode suspension was then drawn out with a sterile syringe (Needle No. 20) or a sterile glass dropper from the tube by keeping the tubes in a 10 ml beaker containing 2 ml sterile water. The whole process was done on a laminar flow table top.

Subculturing was done after 45 days. Five ml sterile water was syringed out, on to the infested carrot disc and the flask shaken gently for 2 minutes. The resultant suspension was then drawn out and three drops (containing 300-400 nematodes) were syringed out on to each new sterile carrot discs.

Subculturing could be done also successfully by taking out the infested carrot disc with the help of a flamed pair of forceps and cutting into several 4-5 mm bits in a sterile petri dish. Each of the pieces was then transferred on to fresh carrot discs. Such bits yielded 400 to 500 nematodes. The above two methods recorded no contamination and also avoided sterilisation of nematodes at every subculturing.

Discolouration of the carrot discs was taken as a sign of multiplication of the nematode. Such culture discs could be kept upto 2 months.

At every subculturing, nematodes extracted from carrot discs were inoculated on to coconut sprouts raised from husked nuts (Koshy and Sosamma, 1978). Inoculated roots

recorded characteristic lesions, rotting and very high populations indicating that the nematodes ability to reproduce on coconut roots has not been altered by culturing on carrot discs.

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