J. Biol. Control, 12(1): 63-66 , 1998

Research Note

A modified method for mass multiplication of *Pasteuria penetrans* (Thorne) Sayre & Starr

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ABSTRACT: A simple method to mass multiply *Pasteuria penetrans* (Thorne) Sayre & Starr an obligate parasite of plant parasitic nematodes, was worked out by modifying the method defined by Stirling and Wachtel (1980). With the increase in seed rate/pot, there was an increase in root content/pot and number of *Meloidogyne incognita* (Kofoide & White) Chitwood females which in turn contributed to an increase in number of infected females and spore number/g root. This method not only enhanced spore production efficiency/pot but also reduced the inherent disadvantage of maintaining and handling a large number of plants under individual pot (*in vivo*) conditions.

KEY WORDS: Eggplant, mass multiplication, *Meloidogyne incognita*, *Pasteuria penetrans*

Pasteuria penetrans (Thorne) Sayre & Starr is an obligate bacterial parasite on several plant parasitic nematodes and it has been identified as potential biocontrol agent (Stirling, 1991). However, its mass production and commercial use are seriously limited by its obligate nature. Although, Stirling and Wachtel (1980) defined *in vivo* multiplication method for *P. penetrans*, the inherent disadvantage of maintaining and handling a large number of plants under individual pot (*in vivo*) conditions was commonly encountered. The present study, aimed at improving this method of mass production by simple means.

Earthen seed pans (40cm diam) were filled with 3 kg of autoclaved (1.1kg/cm^3) soil mixture (2 parts each of soil and sand, and 1 part of compost), followed by thorough mixing of 10g root powder laden with *P. penetrans* spores @ 2.2 x 10^6 spores/g root powder and sowing of eggplant seeds cv. Pusa Purple Long, @ 2.5, 5.0, 7.5 and 10.0 g/seed pan. Each seed rate was replicated 4 times. Ten days after

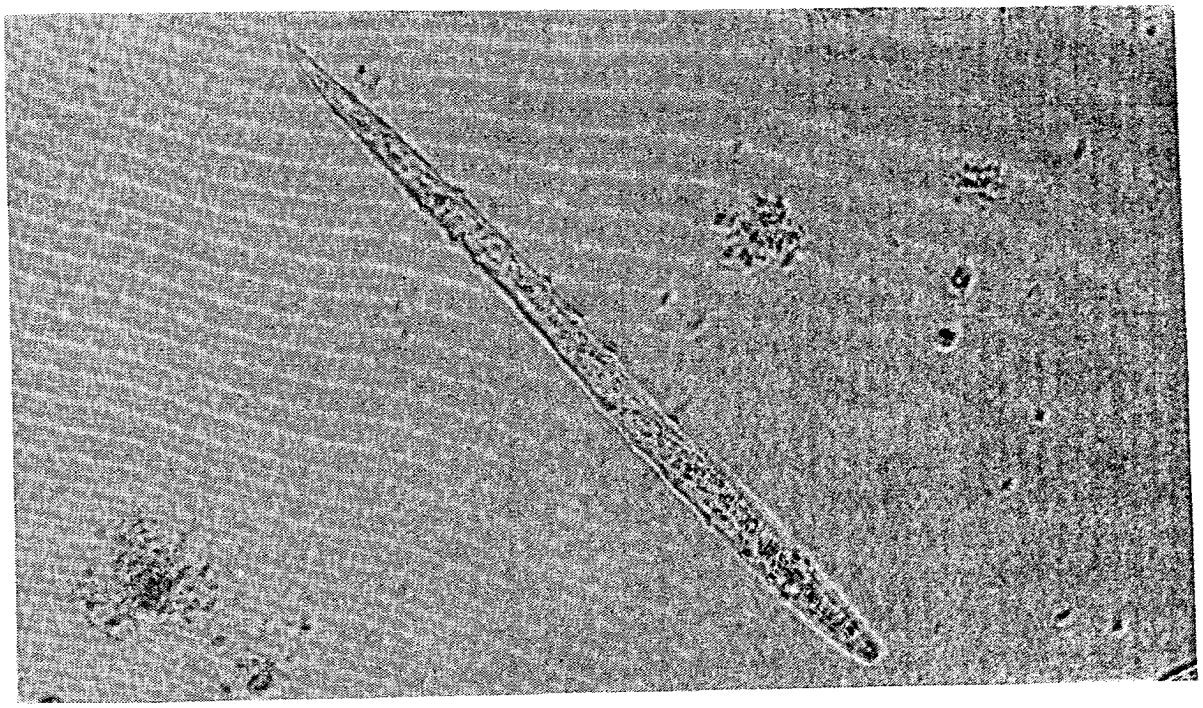
emergence of the seedlings, freshly hatched second stage juveniles (J2) of *Meloidogyne incognita* (Kofoid & White) Chitwood race 1 were inoculated into each pot @ 4 juveniles/g soil.

The plants in seed pan (nursery) were regularly given 50 ml of 20 per cent Hoagland solution at weekly interval for 6 weeks for better root growth and plant vigour (Fig. 1). After 60 days, the plants were carefully uprooted, cleared of soil with water and cut at base of the stem. The healthy and galled roots were air dried, ground to fine powder in mechanical blender and weighed. The spore number/g root was determined by crushing the infected females (collected from 10g of fresh roots) in sterile water and counting the spore number with a haemocytometer. Further, same amount of fresh root was air-dried and the dry weight was recorded to arrive at spore number/g dry root weight. The root powder per pot was calculated accordingly. Further, 0.1 g of root powder from each pot was placed in petri - plates containing 10 ml aqueous suspension of freshly hatched juveniles of *M. incognita* race 1 (@100 J2/ml) for 48 h with intermittent shaking. Two ml of aliquot containing approximately 200 J2 was pipetted into petri-plates and the number of encumbered spores/juvenile were counted randomly for 20 nematode juveniles at 200x (Fig. 2). For each sample, it was repeated 5 times. The total number of spores/0.1g root powder was calculated based on the number of encumbered spores/ juvenile multiplied by the number of juveniles/10 ml of aqueous nematode suspension.

The final number of spores/pot was arrived at by multiplying the number of spores/g root with total root weight/pot.

The data presented in Table 1 indicated that, as the seed rate/pot increased, there was an increase in root weight/pot, number of *M. incognita* females and spore number/ g dry root weight. Further, the number of infected females significantly increased with the increase in seed rate, indicating greater number of bacterial encumbered juveniles developed into females. All these parameters effectively contributed to the total turnover of the spore laden root powder. The spore laden root powder was termed as parasite preparation by Stirling and Wachtel (1980). The use of higher seed rate and Hoagland nutrient solution resulted in higher root content in a given amount of soil, favouring higher root penetration by parasitized (encumbered) nematodes and their subsequent development into infected females thus increasing the spore production efficiency.

Stirling and Wachtel (1980) obtained $2x10^9$ spores/g root while in our studies we could record a maximum of 18.8-$19.8x10^9$ spores/g of galled root indicating that greater the availability of root mass, higher the per cent of parasitized (encumbered) nematodes that developed into infected females/g root. It suggests that the bacterium could be mass multiplied at higher rate (in terms of spore laden root powder) within a time span of 60 days in a given amount of soil, by increasing the seed rate, nematode inoculum and plant nutrition (Hoagland solution). Further, it is concluded that bigger seed pans with



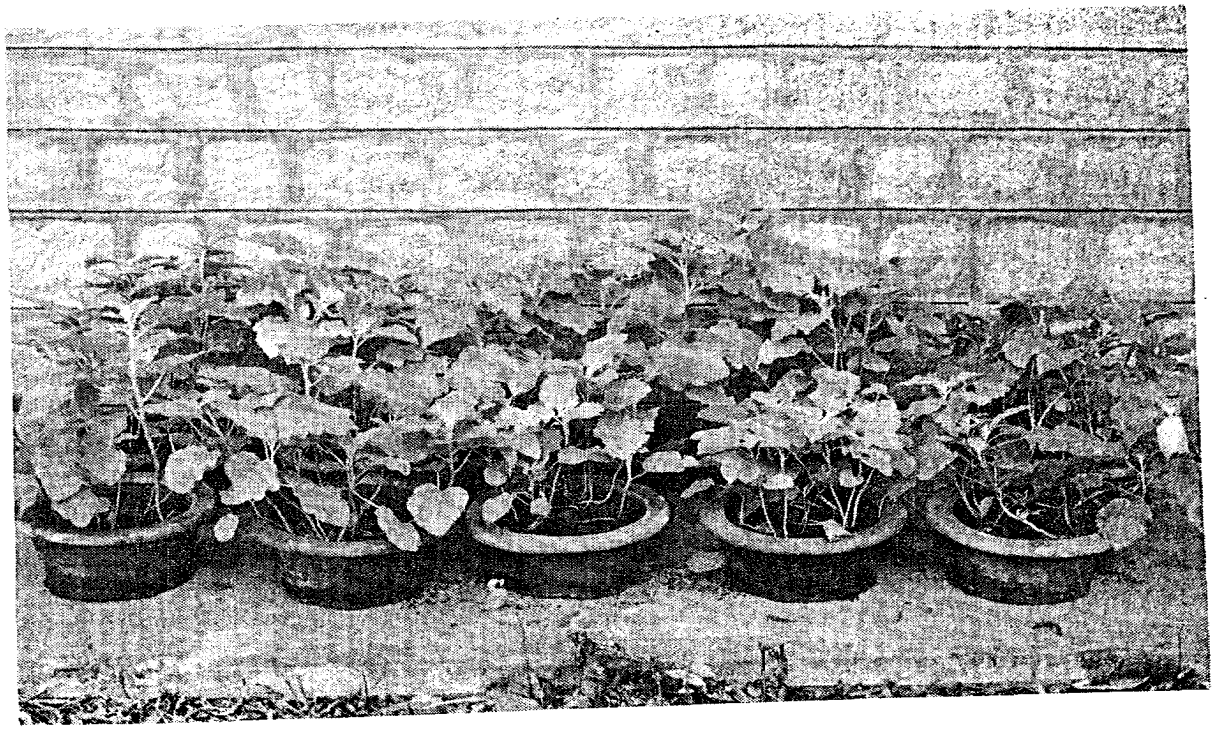


Fig. 1. Mass production of P. penetrans on M. incognita infested eggplants in earthern seed pots

Fig. 2. Second stage juvenile of M. incognita encumbered with P. penetrans spores

Table 1. Effect of seed rate of eggplant on root weight, *P. penetrans* spore number and its production efficiency

Seed rate / *pot (g)*	*M. incognita* galled root	Females/*g*	Dry root weight / pot (g)	Spores/*g* dry root powder	Total spore number / pot	Production efficiency
	Total	Infected				
2.5	226 ± 11	110 ± 8 (48.67)*	38.5	8.6×10^{7}	3.35×10^{9}	1.52×10^{2}
5.0	389 ± 19	198 ± 11 (50.89)	88.6	10.2×10^{9}	9.03×10^{11}	4.10×10^{4}
7.5	494 ± 28	279 ± 14 (56.47)	129.7	19.8×10^{9}	2.56×10^{12}	1.16×10^{5}
10.0	602 ± 18	398 ± 16 (66.11)	186.5	18.8×10^{9}	3.50×10^{12}	1.59×10^{5}
CD (P=0.05)	-	-	2.25	1.88	0.98	0.42

* Values in parentheses indicate per cent infected females

proportionately higher seed rate of eggplants may also be effectively employed for rapid and large-scale production of *P. penetrans*.

ACKNOWLEDGEMENTS

The authors thank the Director, Indian Institute of Horticultural Research, Bangalore for providing the facilities during the study.

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