MICROWAVE TREATMENT OF *PASTEURIA PENETRANS* PARASITE PREPARATION FOR SELECTIVE ELIMINATION OF UNDESIRED MICROORGANISMS

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Summary. A *Pasteuria penetrans* spore laden parasite preparation, raised from peanut pod hulls in a field naturally infested with the bacterium and *Meloidogyne arenaria* (race 1), was exposed to microwave (MW) radiation to eliminate undesired microorganisms. MW radiation was given at medium, medium high or high levels for 1 or 2 min. Treatment of the parasite preparation at high radiation level for 2 min completely eliminated the undesirable fungal propagules, without significantly impeding the viability of *P. penetrans* endospores.

The bacterial parasite, *Pasteuria penetrans* (Thorne) Sayre *et* Starr, is a promising biocontrol agent of root-knot nematodes, *Meloidogyne* spp. *Pasteuria* spp. are obligate parasites and are currently mass produced on nematode hosts in potted plant cultures following the technique devised by Stirling and Wachtel (1980). *Meloidogyne arenaria* (Neal) Chitw. attacks both roots and pod hulls of peanuts. It is possible with existing technology to collect large amounts of *P. penetrans* endospores from peanut fields simply by collecting the hulls after peanuts have been shelled. Peanut hulls are then ground to produce an endospore-laden powder. However, this method is prone to contamination with other microorganisms, especially pathogenic fungi, which may hinder the commercial prospects of *P. penetrans*. The objective of this study was to exploit the use of microwave radiation for 'cleaning' the parasite preparation of undesirable microbes, without drastically impeding the efficacy of *P. penetrans* endospores.

MATERIALS AND METHODS

Peanut (*Arachis hypogaea* L., cv. Florunner) shells naturally infested with *P. penetrans* and *Meloidogyne arenaria* (race 1) were collected from a field (Green Acres Agronomy Farm, University of Florida, Alachua County, USA). The shells were air-dried and ground to a fine powder by using a Wiley grinding mill. The powdered preparation was passed through a 150 µm pore size sieve to eliminate coarse particles. The number of *P. penetrans* endospores present in this powdered preparation was estimated as 6.4×10^6 per g using a haemocytometer.

A sample of 100 mg of powder was transferred to each of a series of BPI watch glasses. A set of three watch glasses (replicates) was kept covered in glass Petri-plates and subjected to microwave (MW) treatment (1500 Watts, 2450 MHz, Tappan Appliance, Mansfield, OH) at medium, medium high, or high radiation level. Each treatment comprised two timings, i.e., 1 and 2 min. A set of untreated samples served as control. The three radiation levels were the main treatments and the two timings the sub-treatments. Upon cooling, each sample of 100 mg was processed for fungal assay using a dilution-plating method. The 100 mg of powder was dispersed in 100 ml of sterile distilled water and 0.1 ml of the suspension was poured over 1% potato dextrose agar plates containing 50 mg chlortetracycline hydrochloride, 100 mg streptomycin sulfate and 1 ml Tergitol NP 10 per 1 litre of solution. There were four replicate plates for each sample. The number of fungal colonies was counted after three days of incubation.

A second set of samples was exposed to MW radiation similarly, and assayed for bacterial spore attachment to *M. arenaria* (race 1) juveniles (J_2s). Each treated 100 mg sample was mixed with 40 g sterile sand in a 5-cm-diameter Petri-plate. Five hundred J_2s held in 6 ml water were added to each Petri-plate. After three days, the J_2s were extracted by a sugar flotation method, and a random sample of 20 J_2s from each replicate was examined under an inverted microscope at 400x. The numbers of J_2s with attached endospores and the numbers of endospores attached per J_2 were recorded. Juveniles with attached endospores were inoculated onto tomato plants to test the viability of the spores.

RESULTS AND DISCUSSION

MW treatment of the parasite preparation significantly reduced the number of fungal colonies, which

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Table I. Effect of microwave radiation on *Pasteuria penetrans* parasite preparation.

Radiation level	Exposure time		Mean
	1 min	2 min	
	A. Number of fungal colonies/Petri plate		
Medium	16.91	10.83	13.87
Medium High	17.50	6.25	11.87
High	7.50	1.00	4.25
Untreated control	19.75	19.75	
Mean	13.97	9.04	
C.D. (P = 0.05) Radiation Levels = 3.50, Exposure Time = 2.74, Interaction = 4.95			
	B. Number of endospores attached per J_2 of *M. arenaria*		
Medium	4.91	2.64	3.77
Medium High	3.67	2.62	3.14
High	3.51	2.41	2.96
Untreated control	3.56	3.56	
Mean	4.03	2.56	
C.D. (P = 0.05) Radiation Levels = NS, Exposure Time = NS, Interaction = NS			
	C. Per cent J2 of *M. arenaria* with endospores		
Medium	55.0	56.7	55.8
Medium High	60.0	55.0	57.5
High	60.0	53.3	56.6
Untreated control	65.0	65.0	
Mean	58.3	55.0	
C.D. (P = 0.05) Radiation Levels = NS, Exposure Time = NS, Interaction = NS			

consisted mainly of *Aspergillus* and *Penicillium* species. Comparing the means of radiation levels (Table 1A), treatment at medium, medium high and high levels resulted in ca. 30, 40 and 78 per cent reduction in the number of fungal colonies, respectively, as compared to untreated control. Similarly, the time of exposure to MW radiation also had a significant effect. The untreated control had 19.7 colonies per Petri-plate, a figure that was reduced by 29.3 and 53.2 per cent at 1 and 2 min exposure time, respectively, when values were meaned over the level of MW treatment (Table 1A). The interaction of radiation level and exposure time was also significant (P = 0.05), with the least number of fungal colonies (1 only) formed after the high level of MW radiation for 2 min (Table 1A).

In contrast, MW radiation levels and exposure times had little effect on the numbers of *P. penetrans* spores encumbering *M. arenaria* J_2s (Table 1B), or on the percentage of J_2s with spores (Table 1C). The interaction of the two factors also was not significant. It is, therefore, concluded that MW treatment of the parasite preparation at the high radiation level for 2 min almost completely eliminates the undesirable fungal propagules, while *P. penetrans* endospore attachment is only marginally reduced. The viability of endospores at this level and time of MW exposure was confirmed by inoculating the spore-encumbered J_2s onto tomato plants and observing spore-filled females after 30 days.

MW treatment has previously been tested as a means of destroying plant pathogens in soil (Baker and Fuller, 1969; Ferriss, 1984) and moisture content was reported to be one of the critical factors. Paradoxically, MW treatment proved effective in eliminating fungal propagules in almost dry carriers, like the pod hull powder of the *P. penetrans* preparation in this study. It is speculated that the water content of the fungal propagules is heated up to lethal levels within 2 min, while *P. penetrans* endospores remain viable since they are reportedly more resistant to higher temperatures (Dutky and Sayre, 1978; Williams *et al.*, 1989). Weibelzahl-Fulton *et al.* (1996) used similar treatments to study the role of *P. penetrans* vis-à-vis antagonistic fungi in the suppression of *M. arenaria*. They treated suppressive field soils with a single level of MW radiation and reported that *P. penetrans* endospores can withstand longer exposure periods than fungi.

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