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Trial 05/01: Microbial inoculants and *Pythium aphanidermatum* on cucumbers

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Aim:
To determine the efficacy of microbial inoculant products to cucumber root rot caused by *P. aphanidermatum.*

Methods:
This trial was conducted in a greenhouse at EMAI commencing on the 18th August 2005. Cucumber seeds (cv. *Deena*) where sown into rock wool plugs and seedlings at the 2-leaf stage were later transplanted into 'coco peat *Easyfil'* bags. Plants were watered and fertilised with complete fertiliser as required. Prior to planting bags were watered and endogenous salts were allowed to leach over a 48-hour period. Just prior to treatment applications styrene trays were placed under bags to capture drainage water and prevent cross-contamination (Figure 1). There were six plants per bag and eight replications of nine treatments set out in a randomised complete block design. The treatments are listed in Table 1.

Figure 1. Cucumber plants growing in coco peat media bags with styrene bases to contain excess moisture

Microbial inoculant products were applied at the following rates: 'Fulzyme Plus' @ 2ml/L; 'Product X' @ 3g/L; and 'Product Y' @ 3g/L. Aqueous suspensions of the products were made up in buckets and 500ml was applied evenly over the surface of respective bags. *Pythium* inoculum was prepared from Potato Carrot Agar cultures of *P. aphanidermatum* (PHDS collection # 04/546) that had been previously isolated from a greenhouse cucumber with root rot symptoms. Culture plates were incubated at 30^{o}C for 7 days and then homogenised in sterile distilled water. An estimate of the *Pythium* inoculum concentration was made from serial dilutions that were plated to agar media and then applying the most probable number technique to data. *Pythium* inoculum (500ml [equivalent to 1,500 colony forming units]) was applied seven days after microbial inoculant products. Negative and positive controls for the microbial inoculants and *Pythium* inoculum were included in the trial design (Table 1).

The greenhouse temperature averaged 26^{o}C with maxima and minima of 35^{o}C and 19^{o}C respectively. Plants were observed regularly and permanently wilted plants were recorded and hygienically placed in plastic bags. Their roots were cultured on agar media to confirm the presence of *Pythium*. Final plant survival counts were made on the 4th October 2005. Sub-samples of remaining plants were collected and their roots plated to agar media to determine if *Pythium* was present.

Plant mortality rates at the final scoring were analysed using a Generalised Linear Model, assuming errors to be binomially distributed. A logit link function was used to relate the observed mortality rates to the parameters that could be used for treatment comparisons. Where observations within each treatment had all zero scores, a small value (0.25) was added to one of the replicates to avoid inflation of standard errors of logit transformed means. Treatment comparisons were determined via the logit coefficients using the least significant difference (LSD) test.

Results & Conclusions

No plant growth measurements were taken, as there was considerable variation between bags in growth rates. This was probably due to uneven leaching of salts in the media prior to planting. Plant mortality scores for the individual treatments are listed in Table 1. There was a clear difference between treatments in plant mortality with the 'Fulzyme Plus' treatment providing equivalent control of *P. aphanidermatum* to the negative controls. In contrast, plant mortality was significantly greater in 'Product X' treated bags (Figure 2) than *Pythium* controls. Similarly, plant mortality scores for the 'Product Y' treatment was equivalent to the positive *Pythium* control. These latter two results are difficult to explain since there were no increased mortalities when these products were used without the addition of *Pythium* (Table 1). These results indicate that neither product provided useful control of Pythium Rot of cucumber when used at these rates while 'Fulzyme Plus' provided excellent control of Pythium Rot of cucumber in this experiment.

Table 1: Mortality scores of cucumber plants and the effects of microbial inoculants on *Pythium aphanidermatum*

Treatments	Logit mean	SE	% Mortality
Fulzyme Plus	-5.2515	1.9654	0.52c
Product X	-3.1355	0.7223	4.17c
Product Y	-3.1355	0.7223	4.17c
Fulzyme Plus + *Pythium*	-3.8501	1.0105	2.08c
Product X + *Pythium*	1.6094	0.3873	83.33a
Product Y+ *Pythium*	1.4663	0.3698	81.25ab
Pythium	0.6008	0.3018	64.58b
Neg Control Agar	-5.2515	1.9654	0.52c
Neg Control	-5.2515	1.9654	0.52c

Note: Mortality scores with different letters are significantly different at 5% level

Figure 2. Stunted and wilting plants in the bag on the left were treated with Product X and *Pythium* contrast with the Fulzyme Plus and *Pythium* treatment on the right.

Trial 06/02: Microbial inoculant ('Fulzyme Plus') and *Pythium spp.* on hydroponic lettuce

Aim:
To determine the efficacy of the microbial inoculant, 'Fulzyme Plus' and *Pythium* spp. on lettuce grown by hydroponic production.

Methods:
This trial was conducted at the Horticultural Research Institute, Gosford. A hydroponic production unit was established with 35 independent channels, each with a 100-litre nutrient tank and recirculating solution using the nutrient film technique.

Two cultivars of lettuce where obtained from a commercial seedling producer: Green Oakand Red Mignonette. They were randomly assigned to positions along channels. Each channel contained forty plants (20 x 2 cultivars), plus buffer plants at each end.

The trial design consisted of five treatments: a microbial inoculant ('Fulzyme Plus @ 2ml/L); propamocarb (Previcur ® @ 1.5ml/L) drenched on seedlings prior to transplanting to the channels; a growth enhancer (Hygrozyme ® at the recommended rate in appropriate tanks); a negative control; and a positive *Pythium* inoculum control. All tanks except for the negative controls received *Pythium* inoculum. These five treatments were randomly assigned to channels in seven replicated blocks.

Pythium inoculum was prepared by homogenising agar PDA cultures in sterile distilled water that was applied to appropriate nutrient tanks seven days after the microbial inoculant and plant growth enhancer. Serial dilution of the inoculum suspension and culturing to agar media was used to estimate the *Pythium* concentration (equivalent to 10^6 colony-forming-units per 100-litre tank). Negative control tanks received homogenised suspensions of uncolonised agar.

Plants were grown until maturity and harvested. Tanks were topped up with water and complete nutrients as required. Whole plants were drained free of water and weighed to obtain wet weights. Sub-samples of roots were taken and cultured to agar media to determine their *Pythium* colonisation.

Wet weight data were fitted into the following model:
Weight = fixed (treatment + cultivar + [treatment x cultivar]) + random (block + channel + [channel x cultivar] + error.
All parameters were estimated using the residual maximum likelihood (REML) estimation and the analysis was run on Genstat (VSN International 2003)

Results and Conclusions
'Fulzyme Plus' treated plants grew significantly bigger than those in all other treatments (Table 4). This growth stimulation was independent of *Pythium* inoculum, which had no significant effect on plant wet weights.

Table 4. Effect of biological and chemical treatments and *Pythium* on wet weights of two lettuce cultivars

	Cultivar		
Treatment	**Green**	**Red**	**Means***
'Fulzyme Plus"	468.0	354.2	411.6a
Previcur ®	427.3	320.7	374.0b
Hygrozyme ®	439.7	323.7	381.7b
Neg. Control	407.7	314.4	361.0b
Pythium Control	410.8	313.7	362.2b
SED			12.5
LSD (5%)			25.9
Means*	429.8A	324.6B	

* Means with different letters indicates significant difference at 5% level