

## **Appendix 4. Methodology for application of BCAs through drip irrigation (The University of Reading)**

### *Experiment I*

The irrigation system was set up according to specifications in the Annexure xx. The treatments were

1. Control, where only water was run for a fixed period of time.
2. Spores of *P. lilacinus* mixed in the tank at the start of irrigation
3. Spores of *P. chlamydosporia* mixed in the tank at the start of irrigation.
4. Spores of *P. lilacinus* and *P. chlamydosporia* mixed separately but delivered into the same target plots one after the other

Twenty-four planter bags were filled with 60 kg each of John Innes No.2 compost, and arranged in four rows of six each. Lateral lines were drawn from the main line at one for each row. With online drippers spaced at 30 cm, there were three drippers in each bag. 30 day old seedlings of tomato were planted in the bags midway between every two drippers such that there were two plants in each bag (Figure 7.1). The requirement of spores of *P.lilacinus* was calculated at the rate of 10000 spores per gram of soil, for 60 kg. Likewise, *P.chlamydosporia* was calculated at 5000 spores per gram. The tank was filled with 48 litres of water, calculated at 4 L per bag, and the spores of *P. lilacinus* were added into it, and stirred constantly to give a homogenous suspension. With the lateral lines Lane-1 and Lane-3 sealed off, irrigation was commenced and the spore suspension run through lateral lines Lane-2 and Lane-4. Water samples were collected from the middle dripper in each bag over five minutes, with the first sample taken immediately after switch-on and the second, midway through irrigation. The water in the tank containing the spores was also sampled during irrigation. Similarly, *P. chlamydosporia* was applied through laterals Lane-3 and Lane-4, with the other two sealed off, leaving Lane-4 with a combination of both *P. lilacinus* and *P. chlamydosporia*. For the control in lateral line Lane-, plain water was discharged for a fixed period of time. Finally the lines through which spores were deployed were individually flushed, with and without the distal ends open. The water collected from the final flushing was distributed equally within the treated plots.

The presence of *P. lilacinus* and *P. chlamydosporia* in the water samples was estimated in terms of colony forming units on selective media as described in Ch. 2 paragraph 2.3.4. The flow rate from each dripper was calculated from the volume of

water sampled over 5 minutes. Composite root samples were collected from the 2 plants in each bag. The samples were plated as described in Ch. 2 paragraph 2.3.4 and the presence of *P. lilacinus* and *P. chlamyosporia* assessed in terms of CFU. Statistical analysis of variance was done applying a split plot design where the samples collected at the start and midpoint (Time of sampling) were considered as the whole plot treatments. The position of plots along the lateral lines was considered as replications and the lanes as sub-plot treatments. For the analysis on the flow of water, all four lanes were taken into account. The CFU of the two organisms were separately analysed taking only the two lanes (without the control) into account.

Figure 1 Schematic diagram of a planter bag showing relative positions of drippers, and plants

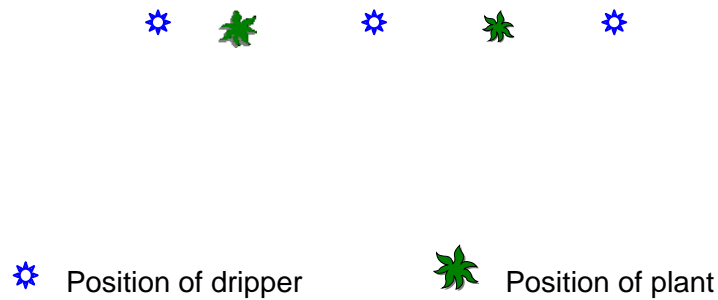
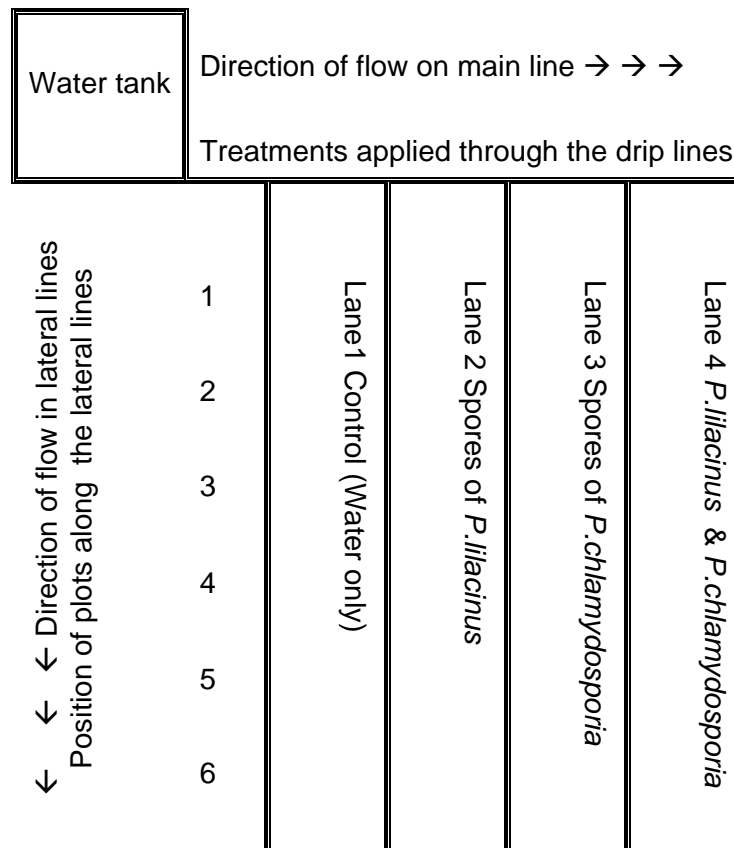


Figure 2 Schematic representation of the layout of experimental plots



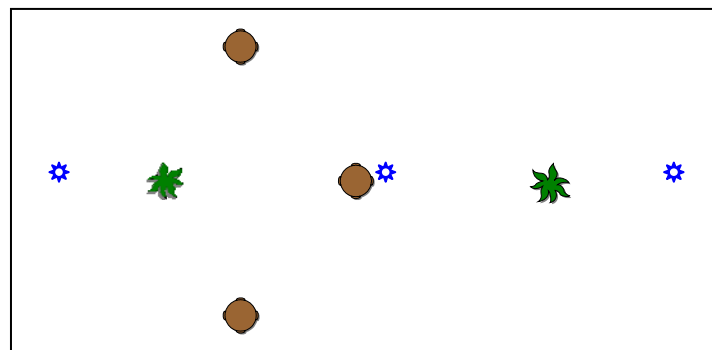
## Experiment II

This experiment was set up similar to experiment I, with 4 bags (replicates) along the laterals. The treatments, in the order of lanes 1, 2, 3 and 4 respectively, were

1. Spores of *Pochonia chlamydosporia* applied in 2 splits as
  - a. 2000/g of soil at the start of the experiment
  - b. 3000/g of soil one month later
2. Spores of *Pochonia chlamydosporia* applied in one dose at 5000/g of soil at the time of 2<sup>nd</sup> dose
3. Spores of *Paecilomyces lilacinus* applied in one dose at 10000/g of soil at the start of the experiment.
4. Control, water only

Sixteen planter bags were filled with 60 kg each of John Innes No.2 compost, and arranged in 4 rows of 4 each. Lateral lines were drawn from the main line at one for each row, at 3 drippers in each bag. Thirty day old seedlings of tomato were planted in the bags midway between every two drippers such that there were two plants in each bag. The requirement of spores of *P.lilacinus* was calculated at the rate of 10000 spores per gram of soil, for 60 kg. Likewise, *P.chlamydosporia* was calculated at 5000 spores per gram. The tank was filled with 16 litres of water, calculated at 4 L per bag, and the spores of *P. lilacinus* were added into it, and stirred constantly to give a homogenous suspension. With the lateral lines Lanes 1, 2 & 4 sealed off, irrigation was commenced and the spore suspension run through Lane-3. Similarly, *P. chlamydosporia* was applied through lateral Lane 1 with 16 litres of water carrying spores at 2000/ g soil, with the other lines sealed off. For the control in lateral line Lane 4, plain water was discharged for a fixed period of time. Flushing of the lines and the use of the washings were done as in experiment I. After a month from the first application, the second split dose of 3000 spores/g of soil for treatment 1 and 5000 spores/g soil for treatment 3 were done as before. Plastic tubes of 20 mm Ø and 15 cm length, tapered at one end were prepared one for each treatment for soil sampling. The sampling points were marked out at 15 cm from the dripper as shown in Figure 3

Figure 3 Schematic diagram of a planter bag showing relative positions of drippers, plants and soil sampling points



Key to symbols

 Soil sampling points     
  Position of plant     
  Position of dripper

The presence of *P. lilacinus* and *P. chlamydosporia* in the soil samples was estimated in terms of colony forming units (CFU) on selective media. Composite root

samples were collected from the two plants in each bag. The samples were plated and the presence of *P. lilacinus* and *P. chlamydosporia* recorded in terms of CFU. The data were analysed statistically using analysis of variance. The CFU from the first dose of treatment 1 and the full dose of treatment 3 were first analysed individually using 'one-way-ANOVA' in randomised block design for a comparison between the 3 sampling points within the treatments. The treatments 1 and 2 were analysed together as in a split-plot design. The counts of nematodes in all the treatments were analysed together using 'one-way-ANOVA'.