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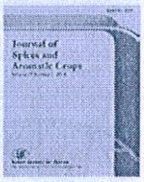


# Effect of biocontrol agents on production of rooted back pepper cutting by serpentine method

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## Abstract

Availability of disease free quality planting material is a major limiting factor in black pepper cultivation. In order to meet the increasing demand and also to create awareness on good agricultural practices for healthy disease free planting material production to farmers, a nursery experiment was started with improved varieties of black pepper by adopting a non-chemical bio-intensive management strategy. Here solarization of potting mixture was the main concern followed my amending the solarized potting mixture with potential bioagents. The experiment was designed in a two factor CRD with four improved varieties and five treatments. Each treatment contains a combination of two bioagents with antifungal and nematicidal properties respectively. The common recommended fungicide Metalaxyl-Mancozeb (0.125%) and nematicide carbsosulfan (0.1%) was used as control. The treatments were incorporated individually into solarized potting mixture and planted with improved varieties used *viz.*, IISR Girimunda, IISR Malabar Excel, IISR Shakti and IISR Thevam, The plants in each treatment were kept for multiplication by serpentine method with proper irrigation and phytosanitation. The results of plant growth and establishment in different treatments, showed that fortification of solarized potting mixture with *Trichoderma harzianum* + *Pochonia chlamydosporia* combination or combination of *Streptomyces* strains (Act 2+9) are significantly superior (35.46% and 21% respectively) for the production of healthy rooted planting material. IISR Malabar Excel and IISR Thevam produced the maximum number of plants from a single node cutting in treatment with *T. harzianum* + *P. chlamydosporia* (T1) (59 nos. and 51 nos. respectively) followed by IISR Malabar Excel with Act 2+9 and Act 5+9 (45 nos. each). So an average of 6-7 plants/month/cutting was produced in the potential treatment while it was only 3-4 plants in control. The advantage of the method is that, after solarization and fortification with respective bioagents, there is no need for further application of any fungicides, insecticides or any other nutrient spray as usually done. Thus the method of soil solarization followed by fortification of either with *T. harzianum*+ *P. chlamydosporia* or combination of *Streptomyces* strains *viz.*, *Ketasatospora setae* (Act 2) and *S. tauricus* (Act9) is found suitable for the production of healthy quality planting material of high yielding varieties to meet the increasing demand of planting material with a C:B ratio of 1:2.

The demand for healthy planting material of high yielding black pepper varieties is on the increase and the country needs large quantity of quality planting material to meet the increasing demand. The present study was aimed to establish a model nursery to produce disease free healthy planting material of improved varieties of black pepper by creating awareness among farmers with good agricultural practices and by adopting non-chemical bio-intensive management strategies.

The experiment was conducted in collaboration with a farmer at Omasserry in Thamarassery Panchayat (Kozhikode district). Initially a nursery shed was constructed (24 m × 20 m) and roofed with white polythene sheet of 100 microns. Single node rooted plants of four released varieties of black pepper *viz.*, IISR Girimunda, IISR Malabar Excel, IISR Shakti and IISR Thevam (indexed for viruses and raised under insect proof conditions at ICAR-Indian Institute of Spices Research, Chelavoor) was used as the source material.

Nursery mixture was prepared by mixing soil, sand and FYM in 1:2:1 and sterilized by solarization. Briefly, the nursery mixture prepared was made into small beds of size 3x1m in a place where there is direct exposure to sunlight. The bed was watered thoroughly and covered with polythene sheet of 100microns and sealed air tight and kept for solarization on 5$^{th}$ November till 25$^{th}$ December 2015.

Biocontrol agents such as *Trichoderma harzianum*, *Pochonia chlamydosporia*, and 3 promising *Streptomyces* sp. *viz.*, *Streptomyces tauricus* (strain Act 9). *Streptomyces* sp. (strain Act 5) and *Ketosatospora setae* (strain Act 2) (Bhai *et al.* 2016) in different combinations were used as growth promoters as well as bioagents for incorporating into the nursery mixture.

The experiment was designed in a two factor CRD with four improved varieties of IISR and five treatments. The five treatments were T1- *T. harzianum* + *P. chlamydosporia*, T2- *Streptomyces* strains 2+9, T3- *Streptomyces strains* 5+9, T4- Metalaxyl-Mz+ Carbsosulfan and T5-control without any amendments. The individual treatments were incorporated with the solarized nursery mixture separately and filled in polythene bags (15 cm × 10 cm) @250 g bag$^{-1}$. *T. harzianum* and *P. chlamydosporia* were made in liquid form with water and added @100 mL (cfu $10^9$ mL$^{-1}$) each to 100 kg potting mixture. *Streptomyces* spp. grown as broth culture in Nutrient broth and 100 mL (cfu $10^{10}$ mL$^{-1}$) mixed with 1 kg vermicompost and grown for 5 days (cfu$10^8$ mL$^{-1}$) ) and applied @1 kg 100 kg$^{-1}$ potting mixture. The treatment imposed poly bags were arranged inside the nursery and planted with single node virus free (indexed) rooted cuttings as mentioned above and were allowed to grow by serpentine method.

When the number of rooted nodes in the serpentine reached around 10, the rooted middle cuttings were cut and separated leaving three plants at the tip and nucleus plant at the end and were kept for establishment to a 3-4 leaf stage in the same nursery. Five plants each were taken from each treatment and observed for biometric growth parameters. The biometric observations were recorded on height of the plant, fresh and dry weight of the plant, number of roots, root length and root biomass. The soil was analysed for the presence of targeted pathogens like *Phytophthora capsici* and nematodes (*Radopholus similis* and *Meloidogyne incognita*), pH and dehydrogenase activity (DHA).

The data were analyzed by using PROC ANOVA procedure of SAS 9.3. Least square means statements were used for mean separation.

After nine months of growth by serpentine method, the variety IISR Malabar Excel and IISR Thevam produced the maximum number of plants in T1 (*T. harzianum* + *P. chlamydosporia* (59 nos. and 51 nos. respectively) followed by T2 (Act 2+9) in case of Malabar Excel (45nos) and T3 (Act 5+9) (45 nos) in case of Thevam, from a single node cutting. An average of 6-7 plants/month/cutting was produced from these varieties with the treatment T1, while it was only 4 plants/month/ cutting in control. In case of IISR Girimunda, the performance was almost the same with all the three bioagent combinations when compared to Metalaxyl-Mz + Carbsosulfan and control. However, IISR Shakti showed comparatively lesser performance with bioagent combinations. The results of the study clearly showed the response of varieties to bioagents. In all cases, the number of plants produced with Metalaxyl-Mz + Carbsosulfan (T4) was comparatively lesser when compared to control (Table 1). The root system was also healthy in all treatments except for control where the root was not profusely grown. No disease of any kind was observed in any of the plants. Though there is no difference between varieties in fresh weight of the plant, the dry weight (Fig. 1) is significantly superior in treatment with *Streptomyces* strains (Act 2+9) and is at par with *T. harzianum* and *P. chlamydosporia*. Not much difference was observed in the number of leaves between treatments but, there is difference in the height of the plants (Fig. 2) where bioconsortia showed increased height when compared to Metalaxyl-Mz. + Carbsosulfan and control. Difference was observed in number of roots, root length, and root fresh and dry biomass (Tables 2 to 5). Since the nursery mixture was solarised and irrigation was limited to once in two days, there was no incidence of soil borne infections caused by *Phytophthora capsici*, *Sclerotium rolfsii* or nematodes (the common diseases otherwise observed in nurseries), in any of the treatments including control (Table 6). The pH of the soil in untreated plants ranged from 4.55-5.66. It is interesting to note that the pH of the soil is raised to neutral in treatments with *T. harzianum* + *P. chlamydosporia* where it ranged from 6.86-7.63. In the case of *Streptomyces* combinations the pH ranged from 4.85 to 6.85 (Table 6) and the dehydrogenase activity which reflects the total oxidative activity of soil microflora, (Liang *et al.* 2014) was unaffected by the incorporation of bioagents like *Trichoderma*, *Pochonia* or *Streptomyces* sp. (Table 6). So without the addition of external nutrients, the micro flora enriched solarized mixture supported the growth of plants as well as prevented the incidence of infection caused by nematodes, *Phytophthora* or any other soil borne pathogens of black pepper. This may be due to the increased microbial activity either through the production of IAA or other growth promoting traits including siderophore production which is observed in the case *Streptomyces* strains (Suseela Bhai *et al.* 2016).

Thankamani *et al.* (2005) reported the effect of *Pseudomonas fluorescens* (IISR-6) and *T. harzianum* (P-26) on the growth of black pepper rooted cuttings in the nursery. However, no

**Table 1.** No. of cuttings produced in nine months from a single plants

| Treatments | IISR Girimunda | IISR Malabar Excel | IISR Shakthi | IISR Thevam | Sub plot mean | Increase over control (%) |
|---|---|---|---|---|---|---|
| $T_1$-*T. harzianum* + *P. chlamydosporia* | 36.00 | 59.60 | 23.00 | 51.00 | 42.40 | 35.46 |
| $T_2$-Act 2+9 | 36.00 | 45.60 | 32.00 | 38.00 | 37.90 | 21.08 |
| $T_3$-Act 5+9 | 34.00 | 34.00 | 32.60 | 45.80 | 36.60 | 15.00 |
| $T_4$-Metalaxyl-Mancozeb + Carbsosulfan | 25.40 | 26.40 | 34.00 | 32.60 | 29.60 | -5.43 |
| $T_5$-Control | 23.60 | 29.40 | 41.60 | 30.60 | 31.30 | - |
| Main plot mean | 31.00 | 39.00 | 32.64 | 39.60 | | |
| LSD (P<0.05) | | | | | | |
| VT | 0.87 | | | | | |
| T | 0.95 | | | | | |
| V × T | 1.90 | | | | | |

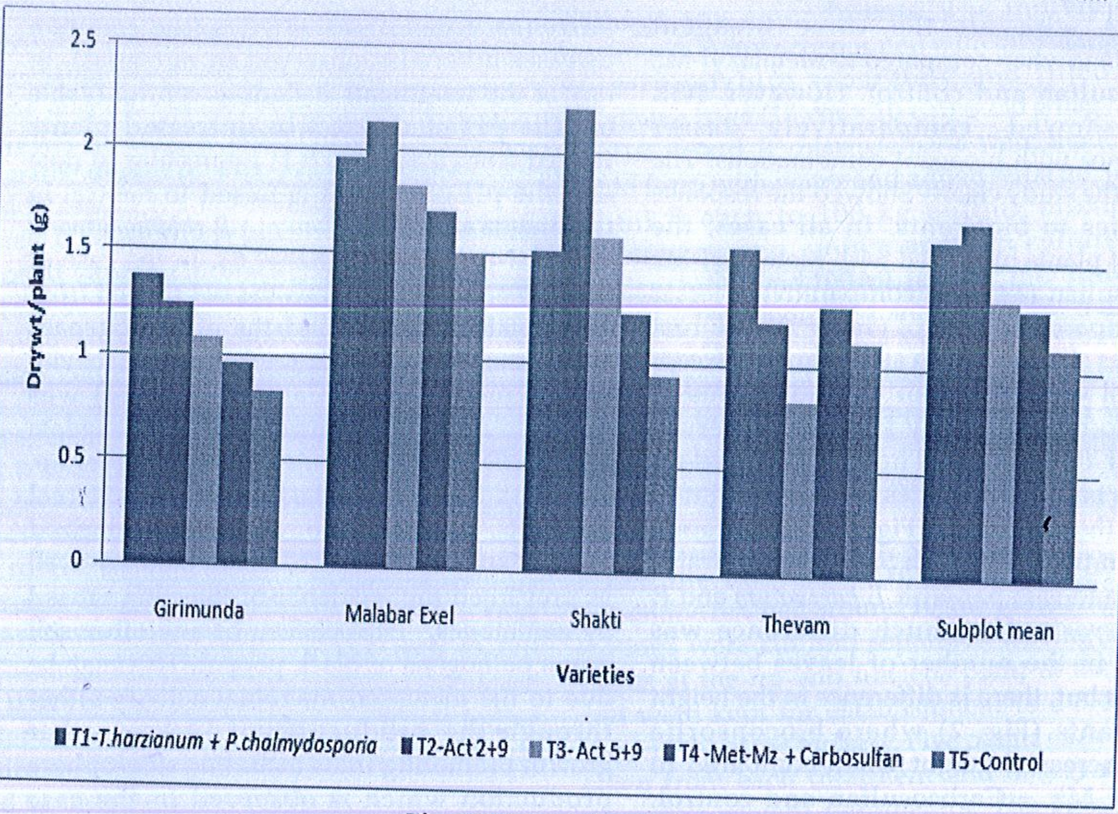


**Fig. 1.** Dry wt (g)/plant

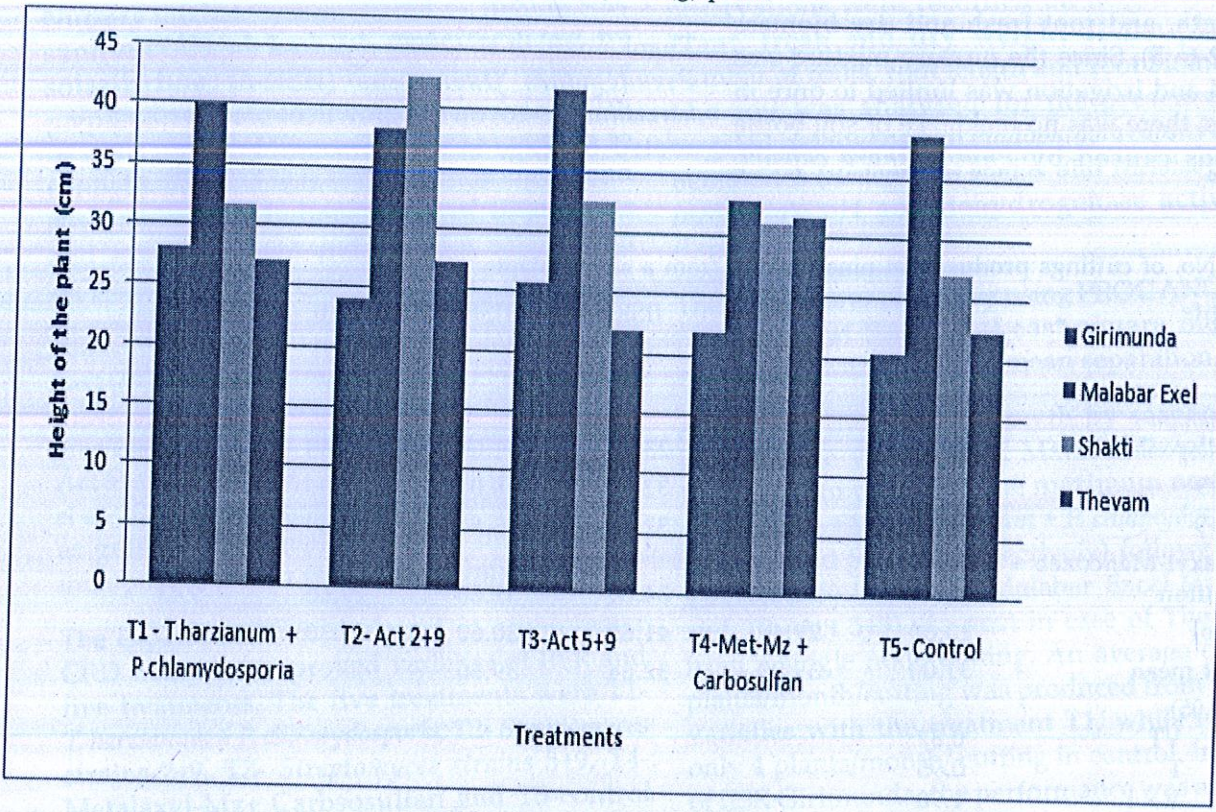


**Fig. 2.** Height of the plants

**Table 2.** Influence of treatments on number of roots

| Treatments | IISR Girimunda | IISR Malabar Excel | IISR Shakthi | IISR Thevam | Sub plot mean |
|---|---|---|---|---|---|
| T1-*T. harzianum* + *P. chlamydosporia* | 7.67 | 8.67 | 4.00 | 12.67 | 8.25 |
| T2-Act 2+9 | 6.00 | 11.33 | 8.33 | 10.00 | 8.92 |
| T3-Act 5+9 | 6.67 | 8.00 | 9.33 | 8.33 | 8.08 |
| T4-Metalaxyl-Mancozeb + Carbsosulfan | 6.33 | 7.33 | 5.00 | 8.00 | 6.67 |
| T5-Control | 8.67 | 6.33 | 5.33 | 7.33 | 6.92 |
| Main plot mean | 7.07 | 8.33 | 6.4 | 9.27 | |
| LSD (P<0.05) | | | | | |
| V | 2.02 | | | | |
| T | NS | | | | |
| V × T | NS | | | | |

**Table 3.** Influence of treatments on root dry biomass (g)

| Treatments | IISR Girimunda | IISR Malabar Excel | IISR Shakthi | IISR Thevam | Sub plot mean |
|---|---|---|---|---|---|
| T1-*T. harzianum* + *P. chlamydosporia* | 0.99 | 0.62 | 0.52 | 0.97 | 0.78 |
| T2-Act 2+9 | 0.93 | 0.98 | 0.57 | 0.54 | 0.76 |
| T3-Act 5+9 | 0.51 | 0.50 | 0.3 | 0.40 | 0.54 |
| T4- Metalaxyl-Mancozeb + Carbsosulfan | 0.47 | 0.54 | 0.37 | 0.51 | 0.47 |
| T5-Control | 0.44 | 0.41 | 0.41 | 0.56 | 0.46 |
| Main plot mean | 0.67 | 0.61 | 0.52 | 0.6 | |
| LSD (P<0.05) | | | | | |
| V | 0.05 | | | | |
| T | 0.14 | | | | |
| V × T | 0.29 | | | | |

**Table 4.** Influence of treatments on root length (cm)

| Treatments | IISR Girimunda | IISR Malabar Excel | IISR Shakthi | IISR Thevam | Sub plot mean |
|---|---|---|---|---|---|
| T1-*T. harzianum* + *P. chlamydosporia* | 31.67 | 36.33 | 19.00 | 22.33 | 27.33 |
| T2-Act 2+9 | 29.00 | 32.67 | 21.00 | 24.33 | 26.75 |
| T3-Act 5+9 | 26.67 | 26.67 | 24.67 | 26.00 | 26.00 |
| T4- Metalaxyl-Mancozeb + Carbsosulfan | 21.67 | 29.67 | 18.00 | 28.00 | 24.33 |
| T5-Control | 18.00 | 26.33 | 28.33 | 24.00 | 24.17 |
| Main plot mean | 25.4 | 30.33 | 22.20 | 24.93 | |
| LSD (P<0.05) | | | | | |
| V | 5.29 | | | | |
| T | NS | | | | |
| V × T | NS | | | | |

**Table 5.** Influence of treatments on root fresh biomass (g)

| Treatments | IISR Girimunda | IISR Malabar Excel | IISR Shakthi | IISR Thevam | Sub plot mean |
|---|---|---|---|---|---|
| $T_1$-*T. harzianum* + *P. chlamydosporia* | 5.37 | 5.83 | 3.53 | 6.70 | 5.36 |
| $T_2$-Act 2+9 | 4.37 | 7.07 | 3.83 | 4.23 | 4.88 |
| $T_3$-Act 5+9 | 2.87 | 4.37 | 5.63 | 3.37 | 4.06 |
| $T_4$- Metalaxyl-Mancozeb + Carbsosulfan | 2.47 | 4.07 | 2.93 | 4.00 | 3.37 |
| $T_5$-Control | 2.97 | 3.47 | 3.30 | 3.97 | 3.43 |
| Main plot mean | 3.61 | 4.96 | 3.85 | 4.45 | |
| LSD (P<0.05) | | | | | |
| V | 0.54 | | | | |
| T | 1.32 | | | | |
| V × T | NS | | | | |

**Table 6.** Effect of treatments on pH and Dehydrogenase activity

| Treatments | IISR Girimunda | | IISR Malabar Excel | | IISR Shakthi | | IISR Thevam | |
|---|---|---|---|---|---|---|---|---|
| | pH | DHA | pH | DHA | pH | DHA | pH | DHA |
| $T_1$- *T. ha* + *P. chal* | 7.38 | 1.21 | 6.86 | 2.72 | 7.30 | 1.98 | 7.63 | 2.05 |
| $T_2$- Act 2+9 | 5.56 | 3.57 | 4.85 | 2.74 | 5.43 | 2.19 | 6.72 | 2.22 |
| $T_3$- Act 5+9 | 5.05 | 3.17 | 6.68 | 3.13 | 5.17 | 1.14 | 5.10 | 1.68 |
| $T_4$- Met-Mz + Carbosulfan | 6.46 | 2.52 | 5.44 | 1.98 | 4.81 | 0.99 | 6.33 | 1.10 |
| $T_5$- Control | 5.64 | 0.94 | 4.55 | 0.96 | 5.66 | 0.53 | 4.86 | 0.61 |
| CD (P<0.05) | 0.10 | 0.09 | 0.09 | 0.02 | 0.12 | 0.17 | 0.09 | 0.22 |

such reports are available for the combined use of *T. harzianum* and *P. chlamydosporia* for the production of rooted plants of black pepper, except for the individual use of *T. harzianum* against foot rot and *P. chlamydosporia* against slow decline diseases. The present study recommend soil solarisation along with the use of *Trichoderma* + *Pochonia* combination or combination of *Streptomyces* strains Act 2+9 for the production of healthy rooted planting material. The effect of solarized potting mixture on growth of black pepper rooted cuttings was reported earlier (Thankamani *et al.* 2007). Since both *Phytophthora* and nematodes are serious threats of black pepper, the treatment combinations are made in such a way that the combination contain one antagonist against *Phytophthora* and another antagonist against nematode. Similar work was reports for the use of consortia in planting material production. Consortia of *P. fluorescens* + *T. harzianum* + *Paecilomyces lilacinus* were used for the production of tomato seedlings for combating nematode infection (Mukhtar 2013) and *P. fluorescens* + *T. harzianum* for the production of nematode free papaya seedlings (Rae 2007). The promotive effects of *Pseudomonas* and *Trichoderma* were quite significant in growth promotion in tomato during nursery and crop growth stages (Kumar *et al.* 2007). Similarly, Mukhtar (2013) reported the biocontrol potential of *Pasteuria penetrans, P. chlamydosporia, P. lilacinus* and *T. harzianum* against *Meloidogyne incognita* in okra.

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