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On farm production of *Trichoderma harzianum* using organic matter

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ABSTRACT: Addition of *Trichoderma harzianum* into organic media like neemcake, coirpith, farmyard manure and decomposed coffee pulp caused an immediate increase in the population upto 3 days. Population increase was observed at all the different proportions of initial inoculum levels tried. It also showed similar trends at different locations. Increase in the total population density of *T. harzianum* in the media suggests that competition by native fungi in non-sterile carrier media was not a limiting factor in colonization of the media by the antagonist. Soil amended with organic materials like neemcake, coircompost, farmyard manure and *Gliricidia* leaves showed better growth and survival of antagonist than in soil alone. The carrier materials like neemcake, coirpith, farmyard manure and decomposed coffee pulp serve as nutrient additives to the crop in addition to inoculum production they support.

Key words: *Trichoderma harzianum*, organic matter, population proliferation

There is considerable interest in manipulating the soil microbial community to achieve the biological control of soil borne plant pathogens (Cook and Baker, 1983; Papavizas, 1985). Biocontrol is primarily linked to a sustained increase in active propagules of the antagonist. Foot rot is the most serious disease affecting black pepper (*Piper nigrum* L.). The disease is caused by *Phytophthora capsici* and is soil borne (Sarma *et al.*, 1994). *Trichoderma harzianum* is widely used for the biological control of *P. capsici* infection in black pepper (Sarma *et al.*, 1997). It is recommended to use along with locally available organic materials like farmyard manure, neemcake coircompost etc. before the onset of monsoon and towards the end of monsoon. If it is possible to multiply antagonists on organic materials so that farmers themselves can prepare sufficient amount of inoculum needed for their plantation. This study was conducted to determine the rate of multiplication of *T. harzianum* stock cultures on organic materials largely used in plantation as organic soil amendments.

MATERIALS AND METHODS

Trichoderma harzianum P26 multiplied on sorghum grain was used as source inoculum. For this, grain was milled to get broken into pieces instead of flour. Approximately 400 ml of water was added to 1 kg of milled sorghum to obtain 40% moisture. About 250 g of this material was taken in polypropylene bags of size 10 × 12 inches and tied with rubber bands. It was autoclaved for 1 hour at 121°C (15lb) and allowed to cool. Spore suspension was prepared from a 4 days old culture of *T. harzianum*, grown on PDA (50 ml) in 250 ml conical flask, using 300 ml sterile distilled water. It was filtered through sterile muslin cloth. Each 1 ml of this suspension approximately contained 1×10^7 colony forming units. Suspension was taken in a sterile syringe and 5 ml was injected into each sterilized bag containing sorghum and mixed thoroughly. The bags were incubated at room temperature (28-30°C) for 15 days. The 15 days old cultures were used as inoculum for various experiments (Prakash *et al.*, 1999).

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Experiments for further multiplication of *T. harzianum* on organic media were conducted at four locations, one in Kozhikode and three in Wayanad districts of Kerala.

Sufficient quantity of *T. harzianum* grown on sorghum grain (1×10^8 cfu/g) was mixed with 5 kg each of neemcake, coirpith and farmyard manure to obtain 1:20 and 1:40 proportions. The moisture of the organic media was approximately 40% and it was kept open in a shade house. It was mixed thoroughly at two days interval and added sufficient moisture. The initial population of *T. harzianum* as well as population after 10, 20 and 30 days was estimated by serial dilution plate technique. There were three replications for each treatment. The temperature developed inside the media was measured using a thermometer in order to see whether it affects population proliferation.

In the second experiment, required quantity of *T. harzianum* was mixed with 50 kg each of farmyard manure in order to get 1:25, 1:50 and 1:100 proportions. It was kept open in a field. There were seven replications for each treatment. Population of *T. harzianum* was estimated at 0 hour and also after 10, 20 and 30 days. In the third experiment, required quantity of *T. harzianum* was mixed with 25 kg of coffee husk and 25 kg of farmyard manure together in 1:25, 1:50 and 1:100 proportions. There were seven replications for each treatment. The population of antagonist was estimated at 0 hour and also after 10, 20 and 30 days. In the fourth experiment, required quantity of *T. harzianum* was mixed with farmyard manure (50 kg) to get 1:50, 1:100 and 1:200 proportions. In one treatment, 12.5 kg of *T. harzianum* was mixed with 2500 kg farmyard manure (1:200) and in another 12.5 kg of *T. harzianum* was mixed with 2500 kg of coffee husk (1:200). In the above treatments, even though the proportion was same, the organic material was taken in huge quantity. There were four replications for each treatment. The initial population of *T. harzianum* as well as population after 10 and 20 days was estimated.

Dried and sieved garden soil was taken. Its moisture holding capacity was determined. 200 g soil was taken in plastic container and moisture content adjusted to 50%. Required quantity of dried organic matter, viz. neemcake, coircompost, farmyard manure and *Gliricidia* leaves were added to the soil in order to get 1, 10, 25 and 50% concentration. To each container 2 ml spore suspension of *T. harzianum* having 2×10^7 spores/ml was added and mixed thoroughly. The container was covered with polythene sheet and tied. It was periodically weighed and water was added, if necessary, to replace the loss in moisture. Population of *T. harzianum* was estimated at 0 hour and also after 25, 45, 75 and 95 days. Population (colony forming units) estimation of *T. harzianum* was done from serial dilutions of 5 g samples of mix in 50 ml sterile distilled water. Subsequently 1 ml is added to 9 ml vials and shaken well. One ml from a suitable dilution is plated in TSM (Elad and Chet, 1983). Log values of number of colony forming units per gram of organic media were taken and analysed by ANOVA. Duncan's Multiple Range Test did the mean comparison.

RESULTS AND DISCUSSION

Multiplication of *T. harzianum* on organic materials used for field application

In this study, rate of multiplication of *T. harzianum* was determind on organic media used for field application. When *T. harzianum* was mixed with neemcake, coirpith and farmyard manure in 1:20 and 1:40 proportions, the population has increased after 10 days. Then it declined slowly after 30 days. The coirpith and farmyard manure showed maximum number of cfu in the 1:20 and 1:40 proportions. The entire media turned slightly greenish when *T. harzianum* sporulated after 10 days. Neemcake was found to be colonized by *Penicillium* sp., which spread the entire media quickly. Hence growth of *T. harzianum* was very less as compared to coirpith and farmyard manure (Fig. 1). The mean temperature in the media was 27.4°C for neemcake, 26.5°C for cirpoith and 26.7°C for farmyard manure.

In the second, third and fourth experiments, where the media was kept open in the field, the colonization was found to be less as compared to the previous experiment in the shade house. In the second and third experiments, 1:100 proportions recorded maximum cfu (Fig. 2 and 3). In the fourth experiment, maximum number of cfu

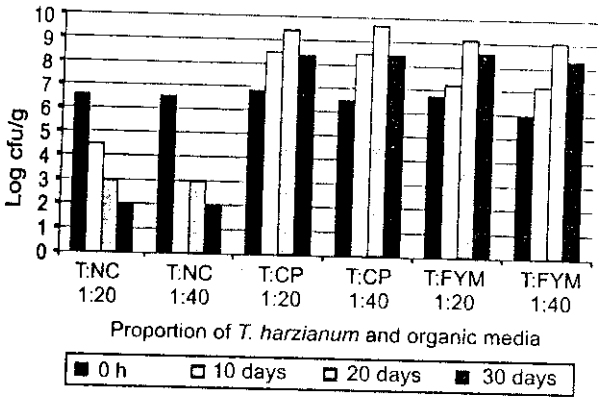

Fig.1. Population proliferation of *T. harzianum* in organic media. *T*, *Trichoderma* grown on sorghum grain; NC, neemcake; CP, coirpith; FYM, farmyard manure

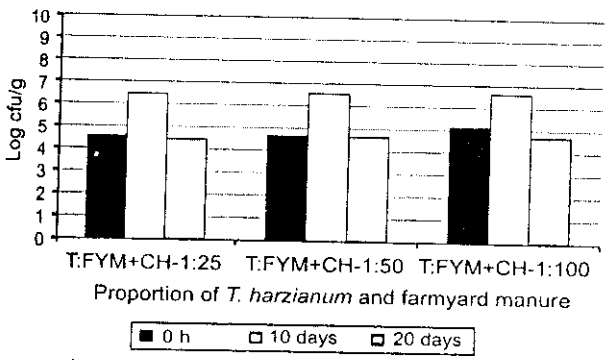

Fig.3. On farm multiplication of *T. harzianum* using mixture of farmyard manure and coffee husk. *T*, *Trichoderma* grown on sorghum grain; FYM, farmyard manure; CH, coffee husk

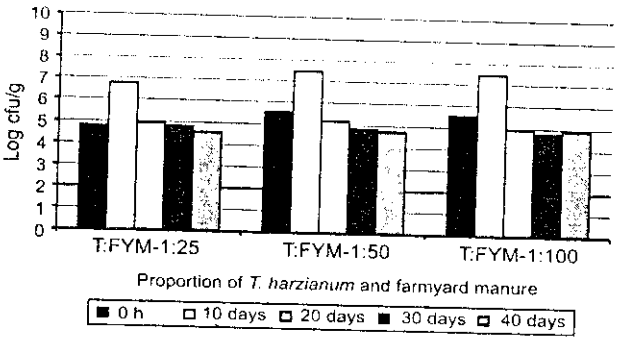

Fig.2. On farm multiplication of *T. harzianum* using farmyard manure. *T*, *Trichoderma* grown on sorghum grain; FYM, farmyard manure

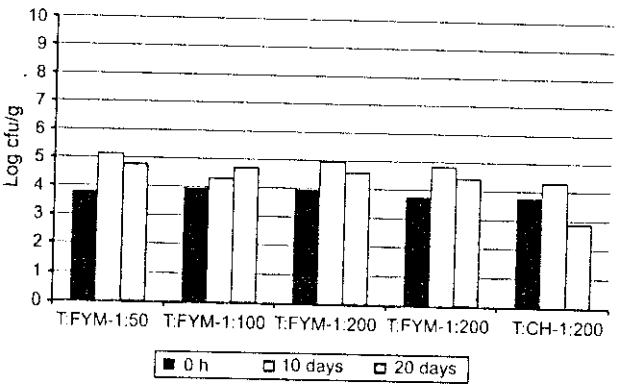

Fig.4. On farm multiplication of *T. harzianum* using farmyard manure and coffee husk. *T*, *Trichoderma* grown on sorghum grain; FYM, farmyard manure; CH, coffee husk

was recorded in 1:50 proportion (Fig. 4). Marois and Locke (1985) recorded similar population increase with *T. viride* in steamed plant growth medium.

Effect of organic amendments on the survival of *T. harzianum*

The moisture holding capacity of the soil was 78.1%. The cfu recorded a sharp increase when organic matter was added. Later it has shown that number of cfu was higher when the percentage of organic matter was in higher proportion (Fig. 5). However, farmyard manure and coircompost showed maximum number of cfu even after 75 days. There was a slow decline in the number of cfu in all the amendments over a period of time. This may be an indication of overpopulation i.e. the number of microbes that can occupy a given amount of soil is limited. Similarly, Beagle-Ristaino and Papavizas (1985) observed increase in the number of conidia when mixed with food base rather than soil alone.

For biocontrol purpose, *Trichoderma* biomass may be applied to the plants or soil as hyphae, chlamydospores or conidia. Hyphae are unlikely to be useful since they will not withstand drying. Lewis and Papavizas (1984) have chosen to produce a biomass dominated by chlamydospores. As conidia are produced much more abundantly than the chlamydospores under same conditions, they are often chosen as the preferred propagules in biocontrol formulations. A successful biocontrol system is one, which is easy and economical to produce, safe, stable in the environment and easily applied during the conventional agricultural practices. One important factor in the formulation

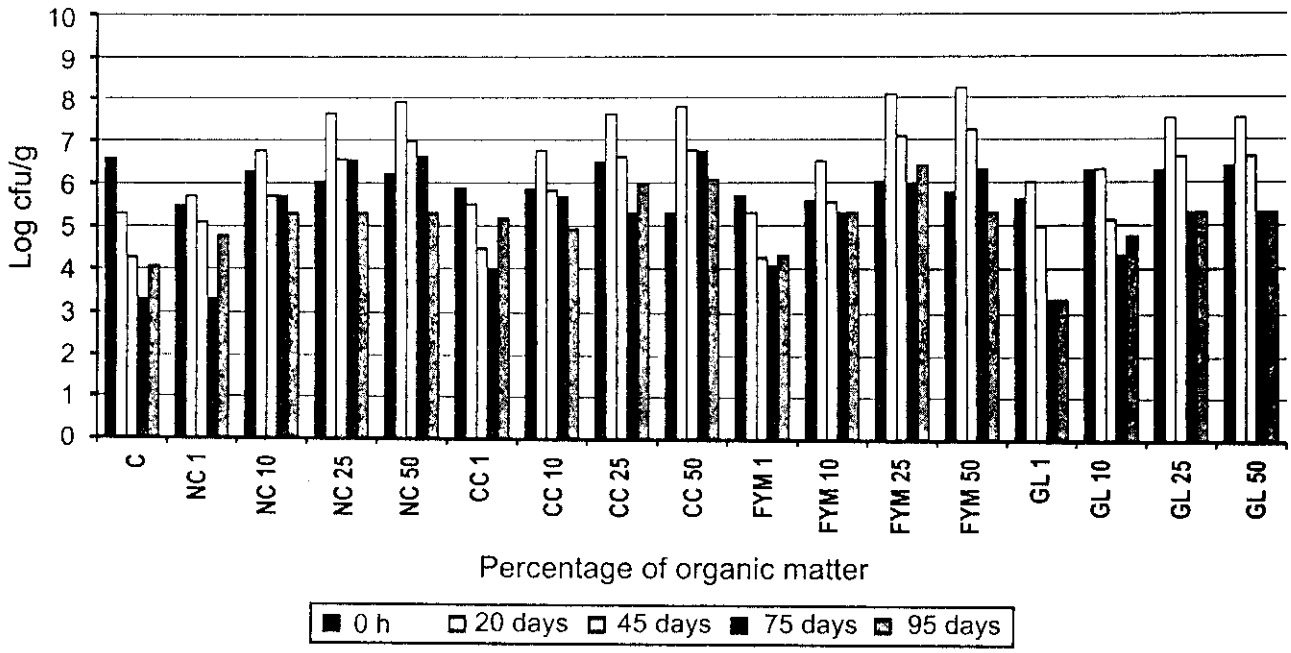

Fig.5. Effect of organic amendments on the survival of *T. harzianum*. C, control (soil alone); NC, neemcake; CC, coircompost; FYM, farmyard manure and GL, *Gliricidia* leaves

is the nutrient status of the support or additives. It is usually necessary for biocontrol agents to proliferate and become established quickly (Tronsmo and Hjeljord, 1998). Organic media available in the plantation areas are excellent source of nutrition for antagonistic fungi like *T. harzianum*. It can be largely used for mass multiplication of biocontrol agents. *T. harzianum* and organic media in the ratio of 1:100 is found ideal. It should be kept away from direct sunlight and rain during multiplication. Sufficient quantity of organic materials should be applied to the soil for better growth and survival of the antagonist. Results presented are useful for comparison of nutritional additives or other formulation for biological control or as a basis to develop eco-friendly system for performance of biological control at different application rates and under varying environmental conditions.

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REFERENCES

Beagle-Ristaino, J.E. and **Papavizas, G.C.** (1985) Survival and proliferation of propagules of *Trichoderma* spp. and *Gliocladium virens* in soil and in plant rhizosphere. *Phytopatholgy* **75**: 729-732

Cook, R.J. and **Baker, K.F.** (1983) The Nature and Practice of Biological Control of Plant Pathogens. The American Phytopathological society, St Paul, Minn., 535pp.

Elad, Y. and **Chet, I.** (1983) Improved selective media for isolation of *Trichoderma* spp or *Fusarium* spp. *Phytoparasitica* **11**: 55-58

Lewis, J.A. and **Papavizas, G.C.** (1984) Production of chlamydospores and conidia by *Trichoderma* spp in liquid and solid growth media. *Soil Biol. Biochem.* **15**: 351-357

Marois, J.J. and **Locke, J.C.** (1985) Population dynamics of *Trichoderma viride* in steamed plant growth medium. *Phytopathology* **75**: 115-118

Papavizas, G.C. (1985) *Trichoderma* and *Gliocladium*: biology, ecology and potential for biocontrol. *Ann. Rev. Phytopathology* **23**: 23-54

Praksh, M.G., Vinayagopal, K., Anandaraj, M. and **Sarma, Y.R.** (1999) Evaluation of substrates for mass multiplication of fungal biocontrol agents *Trichoderma harzianum* and *T. virens*. *Journal of Spices and Aromatic Crops* 8: 207-210

Sarma, Y.R., Anandaraj, M. and **Venugopal, M.N.** (1994) Diseases of Spice Crops. In: *Advances in Horticulture* **Vol.10**, Plantation and Spice Crops (Ed. Chadha K.L. and Rethinam P.) Part 2, p1015-1057. Malhotra Publishing House, New Delhi

Sarma, Y.R., Anandaraj, M. and **Venugopal, M.N.** (1997) Biological control of diseases of spices. In: *Biological control in spices* (Eds. Anandaraj M. and Peter K.V.) p1-19. Indian Institute of Spices Research, Calicut, Kerala, India

Tronsmo, A. and **Hjeljord, L.G.** (1998) Biological control with *Trichoderma* species. In: *Plant-microbe interactions and biological control* (Ed. Boland G.J. and Kuykendall L.D.) p111-126. Marcel Dekker, New York.

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