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Population proliferation and spread of *Trichoderma* spp. in soil under two different delivery systems

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A B S T R A C T

Post application population proliferation (build up) and spread of *Trichoderma* spp., *Trichoderma harzianum* and *Trichoderma viride* in three different types (sterilized, solarised and natural) clay loam soil at 50% water holding capacity (pH 6.5) was studied both under laboratory and field conditions following two types of delivery systems, through organic based (vermicompost + neem cake (90% + 10% w/w)) and seed based (sterilized wheat seed) inocula. The population proliferation and spread of the antagonists were faster when they were applied through organic based inoculums over that of seed based inoculums from the point of application. Similarly population proliferation and spread of the antagonist was more rapid in sterilized soil followed by solarised soil and natural soil respectively. Among the two species of *Trichoderma*, *T. harzianum* have spread faster than *T. viride*.

Keywords: Population proliferation, spread, delivery system, *T. harzianum, T. viride,* clay loam soil

Introduction

Mycoparasitic fungi of the genus *Trichoderma*, particularly *Trichoderma harzianum*, have been used for biocontrol of pathogens of crop plants both soil-borne. A major challenge in implementing biocontrol tool is to maintain stable population of the biocontrol agent so that the beneficial effects are sustained throughout the growing season (Lewis & Papavizas 1984; Papavizas 1985). Soil management practices have pronounced impacts on the population of all types of soil micro-organisms including *Trichoderma* spp. (Mughogho 1980; Munnecke *et al*. 1972). Proliferation and subsequent establishment of biocontrol fungi in soil depends on inoculum age and how inoculum was added in relation to food base. Young mycelial preparation without conidia is much more effective than old one (Lewis & Papavizas 1984). The objectives of the present study were to find out the population proliferation and spread of hypha of *Trichoderma* spp. applied with different forms of inocula.

Materials and Methods

Isolation of Trichoderma spp.

Biocontrol fungus *T. harzianum* and *T. viride* was isolated from the rhizosphere soil on *Trichoderma* specific medium modified by Saha & Pan (1997) using dilution plate technique. Antagonistic potentiality of the biocontrol agent was assessed against many soil borne plant pathogens (Bose *et al*. 2005, Pan 2009). The isolate of *Trichoderma* spp. was maintained on potato dextrose agar (PDA) at 4°C for subsequent use.

Population proliferation of Trichoderma spp. in different soil with different forms of inocula

Population proliferation of antagonist in soil was studied following Bose and Pan (2009).

Inoculum grown on seed and organic substrate was used for soil inoculation. For seed based inoculum wheat seeds were sterilized in autoclave at 1.4 kg/cm^2 for 30 min for two consecutive days and inoculated with 7 day old spore suspension of (10^8 spores/ml) *Trichoderma* spp. In case of organic substrate based inoculum

100 g of vermicompost was amended with 10% neem cake (100+10 w/w) and taken in a double layer polypropylene bag. The material was then inoculated with 7 day old spore suspension of (10^8 spores/ml) *Trichoderma* spp. with the help of a hypodermic syringe at 5 ml/packet. The material was incubated for 7 days in BOD incubator at 261C for preparation of inocula.

Soil was prepared by grinding and passing through 2 mm sieve. The moisture content of the soil was maintained at its 40% water holding capacity and the pH value was also tested. Sterilization of the soil where needed was done in autoclave at 1.4 kg/cm^2 for 1 hr for two consecutive days. The substrate containing inoculum was mixed with 2 kg of soil (sterilized, solarized and unsterilized as the case may be). In the assessment of c.f.u of *Trichoderma* spp. in inoculated soil, sample was collected randomly after amendment and periodically thereafter with the help of a 1cm diameter cork borer from the surface upto a depth of 3 cm at 7 days interval up to 42 days. The soil sample was thoroughly mixed and serially diluted upto a level of 10^{-5} to 10^{-7}. One ml of aliquot from this dilution series was spread on to the surface of Petri plates containing TSM. Observation on number of colonies appeared on TSM after 7 to 10 days of incubation at 26 ± 1^0C, was made and c.f.u was determined as per gram of soil.

Liner growth vis-a-vis spread of the antagonist in soil

Linear growth and spread of *Trichoderma* was studied in unsterilized, sterilized and solarized soil. Natural field soil was grounded and passed through 2 mm screen. Sterilization of the soil where needed was done in autoclave at 1.4 kg/cm^2 for 1 hr for two consecutive days. This soil was spread in an aluminum tray separately for each type of soil to a depth of 10 cm. The moisture content of the soil was adjusted at 40% of water holding capacity with sterile distilled water. The soil was point inoculated at a specific location with one loopful of prepared inocula of *Trichoderma* isolates on sterilized wheat seed. Periodical assay (at 72, 96 and 120 hrs) was made collecting soil samples from different orbits viz. 5, 10, 15, 20 and 25 cm from the point of inoculation. The soil samples were assessed and enumerated for growth in terms of c.f.u content of *Trichoderma* spp. on TSM medium.

Results and Discussion

Population proliferation of Trichoderma spp. in different soil types with different forms of inocula

It was found from the result that when seed based inoculum of *T. harzianum* was added, the population increased in sterilized soil reaching maximum ($5.7x10^6$ c.f.u/g) at 3rd week of incubation and then started decreasing reaching to $2.4x10^6$ c.f.u/g of soil. In case of natural soil the population of *Trichoderma* increased for 2 week ($4.5x10^6$ c.f.u/g) of incubation and thereafter started decreasing (Fig. 1).

It was also found from the results that the ability of *Trichoderma* spp. to proliferate in soil was far better and significant at 5% level when applied through organic food based inoculum grown on vermicompost-neem cake (100+20 w/w) than the seed based inoculum. The population of *T. harzianum* in sterilized soil increased to a maximum level of soil counting $17.6x10^6$ c.f.u/g of soil and in solarized soil up to $11.8x10^6$ c.f.u/g of soil at 4th week of incubation. By the end of 6th week, the population remained at $16.2x10^6$ c.f.u/g and $9.8x10^6$ c.f.u/g of sterilized and solarized soil respectively. In case of natural soil the population also increased with a peak at $10.52x10^6$ c.f.u/g of soil at 3rd week of incubation and then declined to $6.12x10^6$ c.f.u/g of soil at 6th week of incubation (Fig. 1).

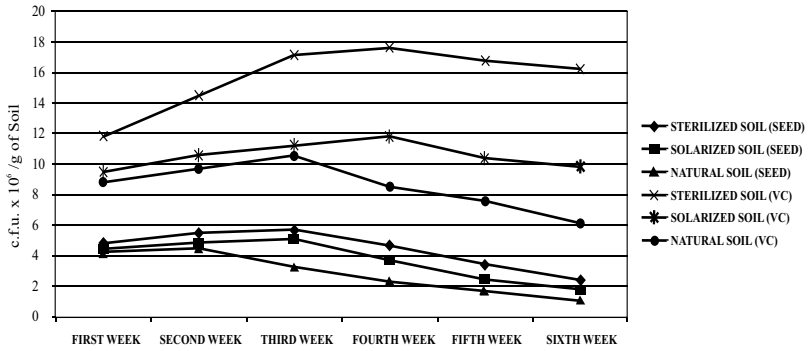

Fig 1. Population proliferation of *T. harzianum (in vitro*) in different state of soil inoculated with different types of inocula

*Each insertion is an average of population of five samples taken randomly

[1]Seed: Inoculum multiplied on sterilized wheat seed (4.08 c.f.u x 10^7/ g of seed)

[2]VC: Inoculum multiplied on sterilized vermicompost+neem cake (100+20 w/w) (8.12 c.f.u x 10^7/ g of substrate)

In case of *T. viride* seed based inoculum proliferated significantly (P=0.05) and reached highest at 2nd week of incubation in sterilized ($4.9x10^6$ c.f.u/g) and solarized soil ($3.56x10^6$ c.f.u/g) whereas in natural soil the population started to decline over the inoculum added to the soil at 1st week ($4.1x10^6$ c.f.u/g) after incubation and decreased up to $0.92x10^6$ c.f.u/g of soil at 6 weeks of incubation. In sterilized soil and solarized soil the population of *T. viride* was estimated to $1.8x10^6$ c.f.u/g and $1.12x10^6$ c.f.u/g of soil at 6th week respectively (Fig. 2).

Population proliferation was also found better when *T. viride* was added to soil through organic food based inoculum (Fig.2). Inoculum grown on vermicompost +20% neem cake resulted maximum population at 3rd week of incubation in all the three soil and there by declined gradually at six weeks of incubation. The result showed that the population of *T. viride* in sterilized soil was significantly higher in 3rd week of incubation with a population of $13.5x10^6$ c.f.u/g of soil than solarized soil ($10.81x10^6$ c.f.u/g) and natural soil ($8.32x10^6$ c.f.u/g). By the end of the 6th week the population remained at $10.52x10^6$ c.f.u/g, $7.2x10^6$ c.f.u/g and $5.2x10^6$ c.f.u/g of soil in sterilized, solarized and natural soil respectively.

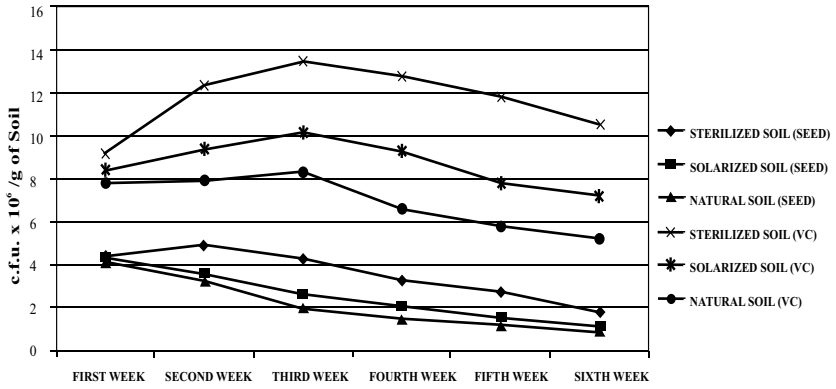

Fig 2. Population proliferation of *T. viride* (*in vitro*) in different state of soil inoculated with different types of inocula

*Each insertion is an average of population of five samples taken randomly

[1]Seed: inoculum multiplied on sterilized wheat seed (4.27 c.f.u x 10^7/ g of seed)

[2]VC: inoculum multiplied on sterilized vermicompost+neem cake (100+20 w/w) (7.68 c.f.u x 10^7/ g of seed)

At field condition the population of *Trichoderma* spp. differed significantly when applied through seed based inoculum and the organic food based inoculum (Fig. 3). Population density of *T. harzianum* significantly increased maximum at 3rd week of incubation. Then population density was at par between 3rd and 4th week of incubation resulting $7.76x10^4$ c.f.u/g and $7.6x10^4$ c.f.u/g of soil respectively. When seed based inoculum was added the population reached peak ($3.05x10^4$ c.f.u/g) at 3rd week of incubation and then declined gradually reaching to a population of $1.5x10^4$ c.f.u/g of soil.

In case of *T. viride* population was also significantly greater in soil amended with vermicompost+20% neem cake than seed based inoculum. Population reached peak ($6.72x10^4$ c.f.u/g) with organic food based inoculum at 3rd week of incubation. But the population of *T. viride* varied insignificantly when added through seed based inoculum up to 3rd week of incubation and then declined to $1.12x10^4$ c.f.u/g of soil at 6 week of incubation (Fig. 3)

[1]Seed: inoculum multiplied on sterilized wheat seed

(*T. harzianum*: $1.24x10^7$ c.f.u/g of seed; *T. viride*: $1.4x10^7$ c.f.u/g of seed)

[2]VC: inoculum multiplied on sterilized vermicompost+neem cake (100+20 w/w)

(*T. harzianum*: $4/75x10^7$c.f.u/g of seed; *T. viride*: $3.9x10^7$ c.f.u/g of seed)

The population of *Trichoderma* spp. significantly varied when applied to different soils with different forms of inocula. The results showed that *Trichoderma* spp. grown on organic substrates showed a rapid and extensive proliferation in soil than *Trichoderma* spp. grown on seed and then the population decreased with time. Population densities of *Trichoderma* in seed based inoculum may have initially increased because of the exudates from seed provided the substrates for their multiplication. The intimate contact between the mycelium and food base might have enabled the fungi to grow relatively unimpeded through soil. Soil colonization by biocontrol agents is greatly

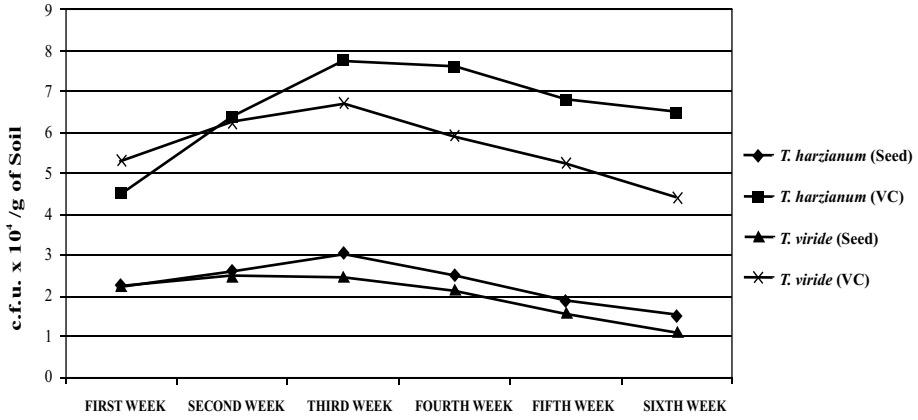

Fig 3. Population proliferation of *Trichoderma* spp. in field soil types with different forms of inocula

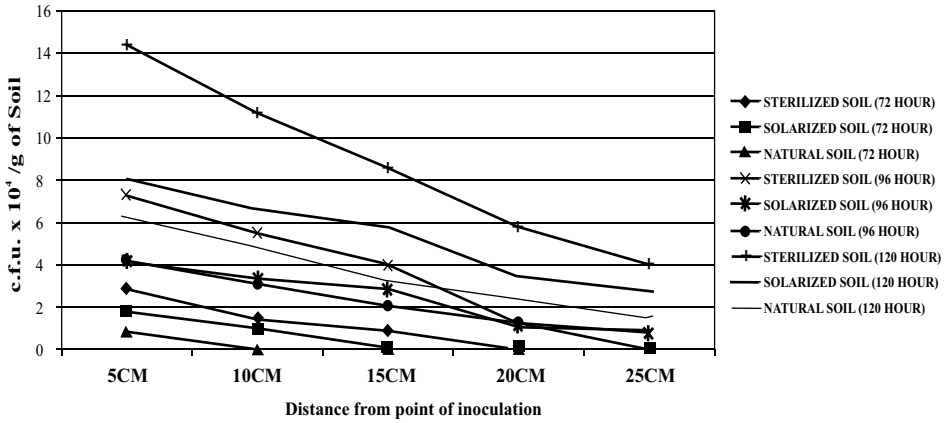

Fig 4. Radial spread of *T. harzianum* in sterilized, solarized and natural soil

affected by the presence of suitable organic substrates in the soil (Garrett 1965; Lewis & Papavizas 1984a). Substrates such as wheat bran (Aziz *et al*. 1997), barley, and bark pellets have been used to incorporate *Trichoderma* into soil with varying levels of effectiveness (Lewis & Papavizas 1984b; Papavizas 1985). Leandro *et al.* (2007) found that *T. hamatum* strain T382 maintains a stable population of 10^3 c.f.u/g soil throughout the growing season when added to field in amended compost in strawberry root. The result also supported that soil treatment with *Trichoderma* based bioformulation increases the population of *Trichoderma* in soil. The indigenous populations of *Trichoderma* present in the soil may also boost up due to nutrient availability through organic substrates. In the present experiment survival and proliferation of *Trichoderma* spp. in field condition also varied with respect to inoculum added. In soil, it has been shown that species like *T. harzianum* can be promoted by substrates with a high C:N ratio (Kwasna *et al.* 2000). Papavizas (1981) reported that *T. harzianum* does not survive well in the rhizosphere of bean and pea seedlings when the seeds are coated with the conidia and when the conidia are applied directly to the soil at 1 day before planting. The young hyphae of *Trichoderma* and other antagonists have a unique ability to proliferate in soil, may be due to their resistance to soil fungistasis (Lockwood 1977).

Radial spread of Trichoderma spp. in different soil (in vitro)

Results on the spread of the antagonist through biomass prepared on vermicompost-neem cake in sterilized, solarized and natural soil condition showed that after 72 hrs of inoculation the hypha of *T. harzianum* reached to distance of 5 cm from the point of inoculation in all types of soil (Fig. 4). In sterilized and solarized soil mycelia of *T. harzianum* spre.ad and proliferated up to 25 cm within 96 hrs and in natural soil it took 120 hrs to reach 25 cm. The population density was very low in non-sterilized soil counting only $1.46x10^4$ c.f.u/g of soil while in sterilized soil and solarized soil contained $4.02x10^4$ c.f.u/g and $2.72x10^4$ c.f.u/g of soil at 25 cm apart from the point of inoculation with *T. harzianum* inoculum.

T. viride also followed the similar pattern of result though hyphal spread and proliferation of the population was low compared to *T. harzianum* (Fig. 5). In natural soil *T. viride* reached at 5 cm within 96 hrs and even at 120 hrs it only reached up to 20 cm from the point of inoculation counting to $0.88x10^4$ c.f.u /g of soil. Whereas in sterilized soil, *T. viride* reached up to 10 cm within 72 hrs and the population density

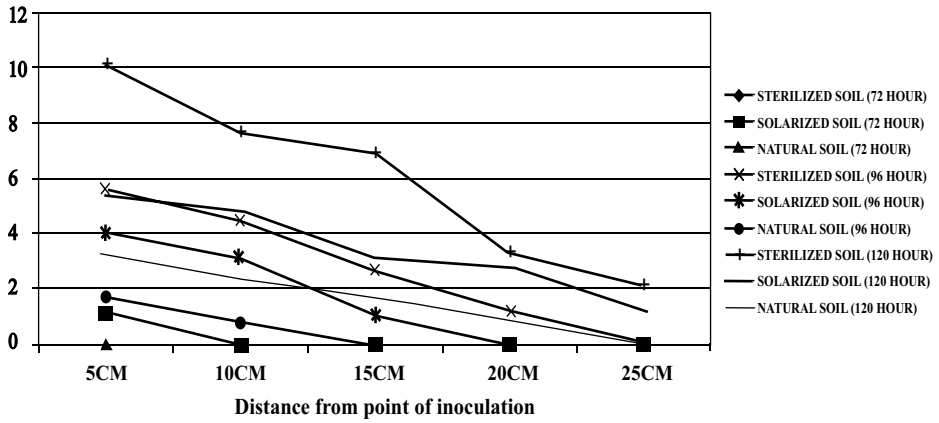

Fig 5. Radial spread of *T. viride* in sterilized, solarized and natural soil

reached up to $2.12x10^4$ c.f.u/g of soil in 120 hrs at 25cm distance from the point of inoculation. In solarized soil almost similar kind of result was acheived giving a population density of $1.2x10^4$ c.f.u /g of soil in 120 hrs at 25 cm.

Lewis and Papavizas (1985) reported that for the fast growing species of *Trichoderma* and *Gliocladium*, hyphal biomass resulted in significant proliferation when added with traces of food base. Kundusen and Bin (1990) found that radial growth rate and hyphal densities are quantified for hyphae originating from alginate pellets containing hyphae of the biocontrol fungus *T. harzianum*. In the present study addition of *Trichoderma* inocula to soil with organic food base media had given significant effect on spread of *Trichoderma* in soil.

It was also found that *T. harzianum* spread faster than *T. viride* which may be an innate character of the specific species. Sterilized and solarized soil supported the easy spread of *Trichoderma* than unsterilized soil that may be due to less or no competition with other soil microorganisms which was in accordance with Bose and Pan (2009). Wu (1986) observed that under natural conditions, sandy loam soil with suitable water content support better stand and spread of *Trichoderma* application compared to silly loam and sandy clay soil with highest population during winter months.

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