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Research Article

Assessment of Optimum Temperature of *Trichoderma harzianum* by Monitoring Radial Growth and Population Dynamics in Different Compost Manures Under Different Temperature

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ABSTRACT

This study was intended for monitoring the optimum temperature of *Trichoderma harzianum*. Optimum temperature for *Trichoderma harzianum* was estimated by two parameter; one was monitoring the radial growth rate and colony morphology in culture plate at 25°C, 30°C, 37°C and 45°C and second was calculating the population dynamics in inert carrier at 4°C, in shade (31±2°C) and in direct sunlight (42±2°C). In this study we used sterilized compost manures as inert carriers which were as follows: 1) JNMsC (Jatropha seed cake, Neem cake, Mushroom spent, *Cuscuta reflexa*) 2) FYM (Farm Yard Manure) 3) CPP-SUPA and 4) Jatropha seed cake and cow dung (JC). *T .harzianum* hyphal extension grown faster at 25-30°C, slower grown at 37°C and no growth was observed at 45°C after six day of inoculation. Population dynamics was maximum in shade followed by 4°C. In sun light population dynamics was decreased very fast in all substrates. Hence optimum temperature of *Trichoderma harzianum* was found between 25-30°C i.e. approx. 28°C by radial growth and population dynamics.

INTRODUCTION

Fungi in the genus *Trichoderma* are among the most widely commercialized biocontrol agents for soil-borne diseases of crops. Species of *Trichoderma* are present in nearly all soils and other diverse habitats (McCray, 2002; Harman, 2005). Both the quantity and quality of organic matter are important for the survival and efficacy of biocontrol agents (Hoitink and Boehm, 1999). Different organic substrate like Jatropa seed cake, Neem cake, Mushroom spent, *Cuscuta reflexa* and some are Vermi compost, CPP-SUPA, Goat manures and farm yard manure also have been suggested for its multiplication (Saju et al., 2002). Like talc based formulation, compost manures was also used for formulation as an carrier. Compost is a rich source of organic matter. When compost manures used as a carrier for formulation then it also plays an important role in sustaining soil fertility, and hence in sustainable agricultural production.

In addition to being a source of plant nutrient, it improves the physico-chemical and biological properties of the soil (Gamal Abdel- Rahman, 2009).The Population dynamics and survival rate of *Trichoderma* depend on the contents of compost manures and also influence of temperature. The variable performance of *T. harzianum* as a biocontrol agent could be because of the influence of environmental factors that vary in time. According to Magan and Lacey (1988) and Plaza et al. (2003), temperature are the principal abiotic parameters determining the germination and growth potential of micro-organism propagules. No literature is available concerning to optimize the practical use of a biological control agent, it is essential to understand how the physical environment affects the agent's survival, growth, and reproduction (Fravel 1999; Sanogo et al. 2002).The general aim of the study to estimate optimum temperature by monitoring the growth and

population dynamics of *T. harzianum* strains (T 35) under controlled conditions.

MATERIALS AND METHODS

In vitro growth of *Trichoderma harzianum* at different temperature:

Effect of temperature on radial growth

Ability of the *T.harzianum* to grow at restrictive temperature was assessed by growing the cultures on PDA plates at different temperature viz., room temperature, 30°C and 37°C and 45°C. For measuring the radial growth rate, *T.harzianum* was inoculated in triplicate at the center of 90 mm Potato dextrose Agar (PDA) plates. Inoculum was aseptically punched with a cork borer in the form of 5 mm mycelial discs from margin of colonies (Morton and Strouble, 1955). The plates were incubated at different temperatures and the radial growth was measured (in mm) everyday upto 6 days of inoculation. The radial growth was measured everyday upto 6 days of inoculation (Segers and Nuss 2003).

Conidiation

For the evaluation of conidiation, mycelial colonies of T. harzianum were grown at different temperatures i.e., 25, 30, 37and 45°C. 5mm culture disk were inoculated at the center of 90 mm diameter petri plate containing 20 ml PDA media. Observations for the conidiation were recorded from day 1 to 6 (Segers and Nuss, 2003).

Population dynamics of *T. harzianum* in composts at different temperatures

The population dynamics of *T. harzianum was* calculated in four different composts at different temperature i.e., at 4°C, in shade (31±2°C) and in direct sunlight (42±2°C).Composts were used *viz.*, 1) JNMsC (Jatropha seed cake, Neem cake, Mushroom spent, *Cuscuta reflexa*) 2) FYM (Farm Yard Manure) 3) CPP-SUPA and 4) Jatropha seed cake and cow dung (JC).

Substrate preparation

Different compost manures and talcum powder was used as substrate viz., Vermi compost, CPP-SUPA, Goat manure, FYM (farm yard manure) and JNMsC (Jatropha cake (50%) + Neem cake (25%) + Mushroom spent (25%) + Cuscuta reflexa (10g/Rep dry wt.). All organic manures was sterilized with 5% formaldehyde and dried (6-7 % moisture) in shade for 5-7 days. Now all five compost manures were used as carrier for the formulation with *Trichoderma harzianum.*

Preparation of Trichoderma harzianum inoculum for compost manures

Inoculum was prepared from *T. harzianum* (T35). Mycelial disks (4-5 disks of 5mm) of the fungus were

Table.1. Effect of temperature on Colony morphology of *T.harzianum* mycelial

	Temperature			
Days	**25°c**	**30°c**	**37 °c**	**45°c**
1day	White	White	×	×
2days	White	White	×	×
3days	White	White/Light Green	White	×
4 days	Light green	Light Green	White	×
5 days	Green	Green	White	×
6 days	Dark Green	Dark Green	White	×

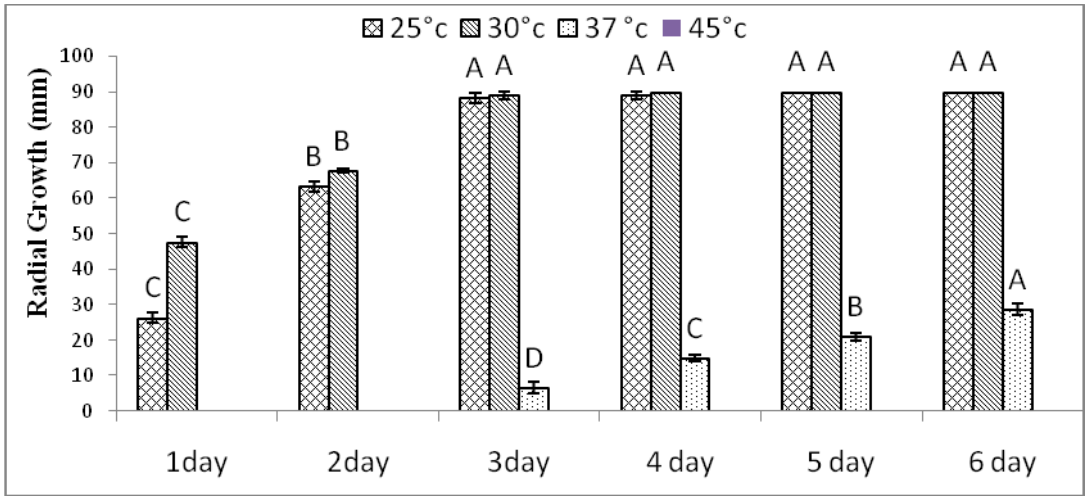


Fig.1: Effect of temperature on radial growth of *T.harzianum* at 25°C, 30°C, 37°C and 45°C after 1D, 2D, 3D, 4D, 5D and 6D on PDA plate. Each value is the mean of three replicates; vertical bars represent standard errors. Means with different letters are significantly different ($P < 0.05$).

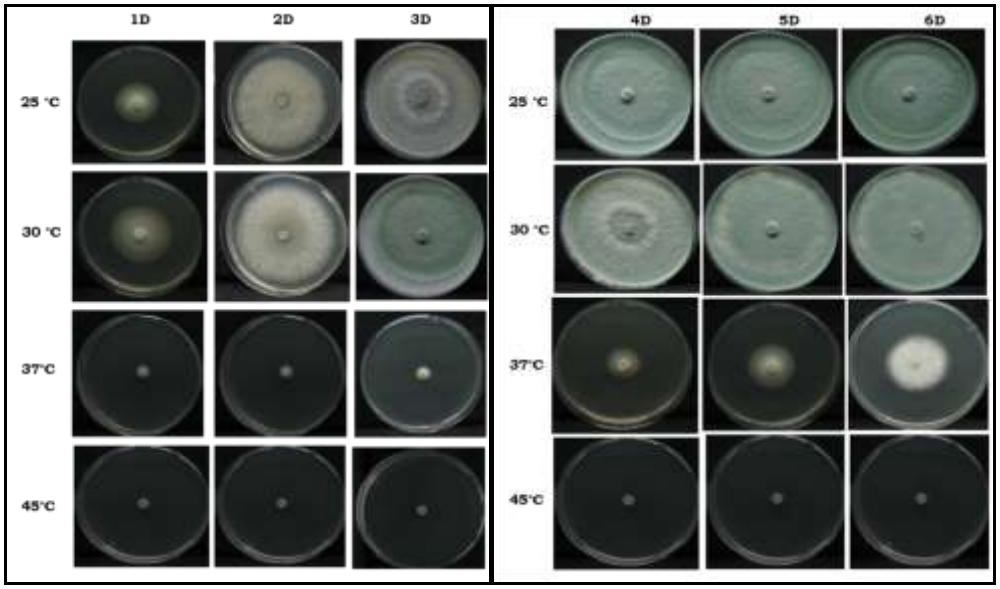


Fig.2. Radial growth and colony morphology of *T.harzianum* at 25°C, 30°C, 37°C and 45°C after 1D, 2D and 3D and b) 4D, 5D and 6D on PDA plate.

introduced in autoclaved sorghum (30 g sorghum in 100 ml distilled water). The culture was incubated for 10 days at 28°C and shaken after each 3 days. After 10 days, culture was dried for 1-2 days in dark then crushed by blender in aseptic condition. *T. harzianum* powder was kept in sterilized poly bag in dark. Each 100 g of compost manures was inoculated with a single 0.25 g dose of sorghum based *T. harzianum* culture and thoroughly mixed in order to spread the inoculum. Colony forming unit (cfu) was counted through serial dilution plating (Ofunne, 1999).

Measurement of population dynamics

The population dynamics of *T. harzianum* in composts was measured at 4°C, room temperature (shade) and direct sunlight from 10 to 60 d by serial dilution plating method (Ofunne, 1999). The plates were incubated at 28°C for 3- 4days. Colony forming unit (cfu) was calculated by using formula:

$$\text{Colony forming unit} = \frac{\text{Number of cells x Dilution factor}}{\text{Inoculum taken}}$$

RESULTS

Effect of temperature stress on the growth of mycelial growth of *T.harzianum*

The ability of the T. harzianum to tolerate temperature stress was assessed by growing the cultures at 25°C, 30°C, 37°C and at high temperature (45°C). Mycelial plugs (5mm) T. harzianum was inoculated at the center of PDA plates and incubated at different temperature. The hyphal extension (from the edge of the plug outwards) was measured at regular intervals. There were no significant differences in the growth rate of the cultures at 25°C and 30°C. The growth rate of *T. harzianum* hyphal extension being 30±1.5 mm per day at 25°C and 30°C. At 37°C radial growth was observed after two days of inoculation, and growth rate was found to slow (Fig.1) i.e., hyphal extension being 5±1.5 mm per day and there was no growth at high temperature (45°C).

Colony morphology

As shown from figure2, the colonies of *T.harzianum* were convex and aerial growth type. Early sporulation was started with the center of the plate at 25°C and 30°C in 3 days and PDA plates were covered with dark green colored mycelial lone in 4-5 days. Mycelial was not covered the entire plate and remained white at 37°C, even up to 6 days. There was no any mycelial growth at 45°C on the PDA plate (Table 1).

Population dynamics of *T.harzianum* in different compost manures and talcum powder at different temperature

Compost, biofertilizers and antagonists have been widely explored as effective and ecofriendly options for controlling plant diseases. An investigation was conducted to study multiplication of *T.harzianum* in compost manures based formulation at 4°C, 28-30°C (shade) and direct sun light (40-45°C).

Compost, biofertilizers and biocontrol agent have been widely explored as effective and ecofriendly options for controlling plant diseases. An investigation was conducted to study the multiplication of *T. harzianum* in different sterilized compost manures (JNMsC, JC, CPP-SUPA, and FYM) at different temperatures. In this experiment, media was kept in sterilized polybags at 4°C, in shade (31±2°C) and in direct sunlight (42±2°C).

Lowest log cfu was found in all above sterilized compost manures in sunlight as compared to shade and at 4°C. Maximum number of log cfu was found in JNMsC (4.0) and CPP-SUPA (4.0) followed by JC (3.8) and FYM (3.9) in 5 days. In sunlight, population dynamics of *T. harzianum* was completely lost within 5 to 10 d in all substrates.

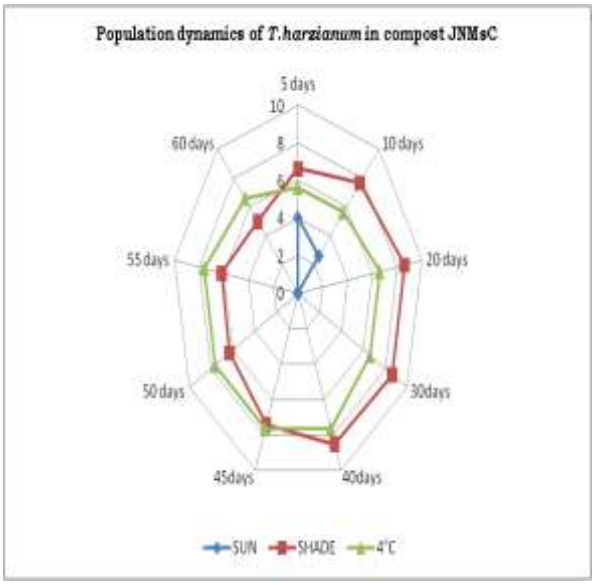
Log cfu was found to be maximum at 4°C in sterilized compost manures. The maximum number of log cfu was found in JNMsC (7.7) and CPP-SUPA (7.6) followed by FYM (6.6) and JC in 40 d. Compost manures, JNMsC and CPP-SUPA showed the maximum number of log cfu after 30-40 days at 4°C that declined slowly after 40 days. Log cfu was found to be maximum in all the above sterilized compost manures in shade as compared to the sunlight and at 4°C. Log cfu was found to be maximum in shade in sterilized compost manures. The maximum number of log cfu was found in CPP-SUPA (8.6) and JNMsC (8.5) followed by FYM (7.3) and JC (7.3) in 40 days. Compost manures JNMsC and CPP-SUPA showed the maximum number of log cfu which was increased after 30-40 d in shade and then declined slowly after 40 d. The population dynamics were found to be highest in the shade followed by 4°C. In sunlight the population dynamics decreased rapidly in all substrates (Fig.3).

DISCUSSION

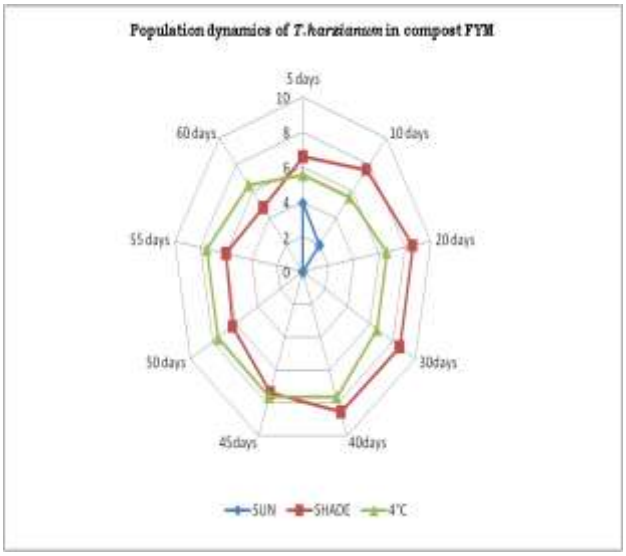
This study represents the first steps toward the estimation of optimum temperatureof *Trichoderma harzianum* through monitoring of radial growth and calculating population dynamics in different compost manures that optimize the growth of the microorganism. This study confirmed that *T. harzianum* was grown better at 25-30°C and very slow grown at 37°C and not grown at 45°C. Greenish colored conidia form within 4-5 days at 25-30°C and remain white upto 6 days at 37°C. Conidia typically form within one week in compact or loose tufts in shades of green at 25-30°C and frequently white on richer media such as potato dextrose agar at 35°C.

Similarly Samuels et al. (2007) reported that, cultures are typically fast growing at 25-30°C, but will not grow at 35°C shown a slight difference in the rate of growth at the temperature 25°C and 30°C as well as statistically significant micro morphological differences. The common incubation temperature for the growth of fungi such as *A. niger* (delille et al. 2004), *Fusarium* sp., *Penicillium* sp. and *Graphium* sp. (Santos & Linardi 2004) was taken at 30°C, *T. virens* was grown at up to 35ºC though with a slight reduction in biomass (Ainon Hamzah, 2012) and *T. atroviride* displayed slower growth at 35°c (Samuels et al., 2002) .*T. aggressivum* f. *europaeum* exhibit very poor growth at 35°C. The mycelium of tested isolates grown the fastest at the temperatures of 25 and 30°C (K. Sobieralski, 2009). Similar results were obtained by Szczech et al. (2008) in experiments conducted in Poland.

The second steps of this experiment towards comparative study of population dynamics of *Trichoderma harzianum* different compost manures at 4°C, shade and in direct sun light. According to J. Kibaki and B.Hau in 2005, the population dynamics of the fungus was affected by both time and the amount of compost in the substrate. These results suggest that application of compost may enhance the proliferation and survival of *T. harzianum*, which could augment the sustainability of biological control following artificial inoculation with the antagonist. The above-mentioned researchers studied at monitoring of population dynamics of *T.harzianum* in different organic substrates but very less literature is available that shown compost manures were used as carrier after sterilization for the formulation of *T.harzianum*.



a)



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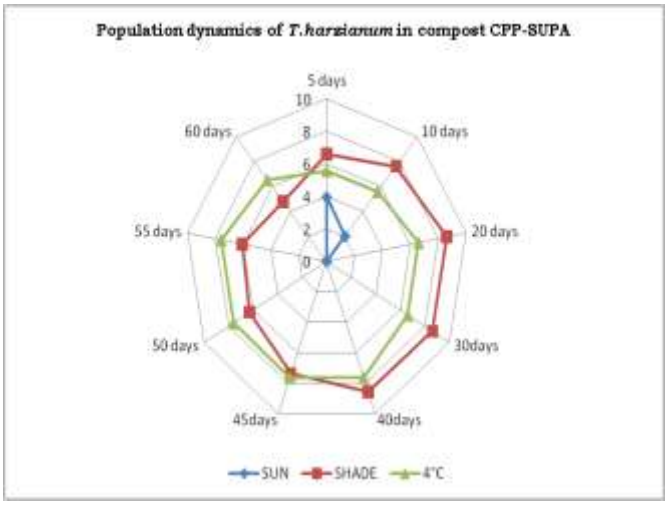

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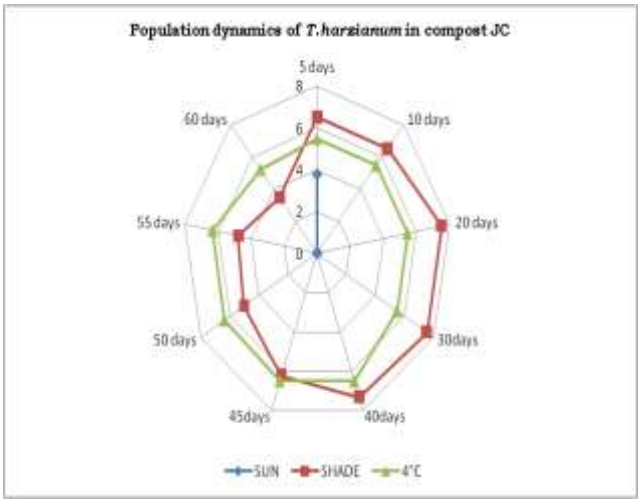

d)

Fig. 3: Population dynamics of *T.harzianum* in a) JNMsC b) CPP-SUPA c) FYM d) JC composts.

Present study confirmed that different organic substrate like Vermi compost, CPP-SUPA, goat manures and farm yard manure (FYM) and JNMsC (Jatropa seed cake, neem cake, mushroom spent, *Cuscuta reflexa*) have been suggested for formulation as carrier as talc based formulation. Population dynamics was found maximum in shade (28-30°C) followed by 4°C. In sun light population dynamics decreased very fast in all substrates.

CONCLUSION

The examined *T. harzianum* showed considerable differences in growth depending on temperature. Both the parameters; the radial growth rate and the population dynamics was turned out that optimal temperature for the *Trichoderma harzianum* mycelium growth was 25 and 30°C.Mycelium growth underwent was decreased rapidly at 35°C and mycelium growth was not observed at 45°C. So this study concluded that *Trichoderma harzianum* was grown fast at 25-30°C approx. 28°C in culture plate as well as compost manures.

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