Journal of Biological Control, 24 (3): 253–256, 2010

Research Article

Studies on the growth and multiplication of *Trichoderma harzianum* and *Trichoderma viride* on different compost manures and their population dynamics *in vitro* and *in vivo* conditions

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ABSTRACT: Compost, biofertilizers and antagonists have been widely explored as effective and ecofriendly options for controlling plant diseases. An investigation was conducted to study *in vitro* growth and multiplication of talc-based *Trichoderma harzianum* and a commercial formulation of *Trichoderma viride* in different compost manures. Optimal compost and vermicompost supported excellent growth of both *T. harzianum* and *T. viride*, however, the efficacy of the mixed formulation of both optimal compost and vermicompost resulted in higher population of both the antagonists and they showed significant increase for up to 45 days after inoculation. Shelf life of both the antagonists was studied *in vitro* and *in vivo* over a period of 120 days. Higher population (cfu g^{-1}) was reported in optimal compost+vermicompost+*T. harzianum*, which was significantly higher than optimal compost+vermicompost +*T. viride*. The maximum population *in vitro* was recorded at 30 days of incubation, whereas it was recorded in 60 days of incubation *in vivo*.

KEY WORDS: Biological control, management, compost, *Trichoderma harzianum, Trichoderma viride*

(Article chronicle: Received: 06.04.2010; Sent for revision: 14.05.2010; Accepted: 23.06.2010)

INTRODUCTION

Biological control of soil borne plant diseases is regarded as an important component of integrated disease management (IDM) system, and it acts as an alternative to various chemical pesticides due to its self sustaining action. *Trichoderma* spp. are most widely used biocontrol agents since they have antifungal and antienduring activities (Zaidi and Singh, 2004). Failure of the antagonist to survive due to shorter shelf life is a major hindrance to consistent field performance. Unlike chemical pesticides, biological control agents need support even after application to get establishment. In the present study, easily available and relatively inexpensive compost / substrates were used to help *Trichoderma* spp. to establish in order to have improved performance.

MATERIALS AND METHODS

Selection of bioagent, compost manure and biofertilizer

Indigenous talc-based *T. harzianum* (AAU DPP 40 Th), commercial biofungicide "*Trichostar*" based on *T. viride* (Super Pesticide Agro (I) Pvt. Ltd. Kolkata), vermicompost (*Vermigold*), optimal compost collected from the Department of Soil Science, AAU, Jorhat, along with biofertilizer rhizobium and phosphate solubilizing bacteria (PSB 5w) were used for the study.

Trichoderma harzianum was multiplied on pre-soaked and autoclaved wheat seeds and supplemented with 12 per cent chick pea powder and was incubated for 10 days at 28±1°C. These were air dried, ground and passed through 50 and 80 mesh sieves simultaneously to obtain pure spore powder. The powder was then mixed with sterilized talc powder [1: 3 (w/w)] containing 1% (w/v) carboxymethyl cellulose (CMC), 0.2% peptone and 2% osmoticant (desiccant) to get the desired concentration of the bioagent in the formulation.

Growth and multiplication of talc-based formulation of T. harzianum *and commercial biofungicide* T. viride *on different compost manure*

Fifty grams of air-dried optimal compost and vermicompost were taken in polypropylene bags separately. Moisture content for organic manure was adjusted to 30% for multiplication of *T. harzianum* and *T. viride*. The bags

were autoclaved at 15 lbs Psi for 30 min and inoculated with two ml suspension of *T. harzianum* (AAU DPP 40) and *T. viride* (*Trichostar*), respectively, and incubated at 28±1°C for 45 days. At 15 days interval, one g of dried sample of each antagonist was used for the enumeration of *T. harzianum* and *T. viride* populations by serial dilution method using *Trichoderma* selective medium in Petri plates. Four replications were maintained for each treatment and arranged in a randomized block design. Treatments included are: optimal compost + *T. harzianum,* optimal compost + *T. viride*, vermicompost + *T. harzianum*, vermicompost + *T. viride*, optimal compost + vermicompost (50: 50) + *T. harzianum*, optimal compost + vermicompost (50: 50) + *T. viride*, talc based formulation of *T. harzianum* alone and a commercial biofungicide *T. viride* alone.

Shelf life of talc-based T. harzianum *and commercial biofungicide* T. viride *in different compost manure* in-vitro

The best compost was recorded individually for *T. harzianum* and *T. viride* and was further selected for the study of shelf life of antagonists. The shelf life of *T. harzianum* (Th) and *T. viride* (Tv) in the best compost obtained was studied at 30 days interval for up to 120 days. One hundred grams of talc-based formulated powder of *T. harzianum* and commercial formulated powder of *T. viride* were added to 500 g packet of compost and mixed properly. Similarly 500 g soil was weighed and taken in a polypropylene bag and autoclaved at 15 lbs psi for 30 minutes and inoculated with 100 g each of *T. harzianum* and *T. viride*. For this, viable populations of *T. harzianum* and *T. viride* in the compost were determined by periodic sampling at 0, 30, 60, 90 and 120 days after storage (DAS). One gram of formulation was drawn aseptically from each polypropylene bag and diluted in 10 ml of sterile distilled water and mixed thoroughly for 20 min in a rotary shaker. Serial dilutions (7-fold) were prepared and 0.1 ml aliquot from 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilutions were spread on PDA plates. After incubating the plates at 28±1°C for 48h, the colony forming unit (CFU) per gram formulation were counted with the help of colony counter.

The experiment was arranged in a completely randomized design maintaining 5 replications for each formulation. The treatment combinations were as follows:

T_1 : Optimal compost + vermicompost (50:50) + *T. harzianum* application

T_2 : Optimal compost + vermicompost (50:50) + *T. viride* application

T_3 : Sterilized soil + *T. harzianum* application

T_4 : Sterilized soil + *T. viride* application

Shelf life of talc-based T. harzianum *and commercial biofungicide* T. viride *in different compost measure* in-vivo

To study the shelf life of *T. harzianum* and *T. viride* in compost manure in soil, an experiment was conducted in soil in pit condition. Pits measuring 45 x 30 x 30 cm were dug out and filled with compost. One hundred grams of talc-based formulated powder of *T. harzianum* and commercial formulated powder of *T. viride* were added to individual pits and mixed thoroughly. Inoculated pits were covered with polythene sheets and sprinkled with water regularly to maintain moisture. Five pits were maintained for each treatment and the population of bioagents was monitored at 30 days interval for four months. The pits were arranged in a factorial complete randomized block design. The treatment combinations were as mentioned in the experiment *in-vitro.*

RESULTS AND DISCUSSION

Optimal compost and vermicompost supported growth of *T. harzianum* and *T. viride* when inoculated individually or in mixed formulation. Mixture of optimal compost and vermicompost (50: 50) resulted in higher population of *T. harzianum* (85.25 x 10^7 CFU) and *T. viride* (78.76 x 10^7 CFU) after 45 days of inoculation. The effect of optimal compost and vermicompost on increasing the growth and multiplication of *T. harzianum* and *T. viride* may be because of increasing C: N ratio (Thirumala Rao and Sitaramaiah, 2000). Chemicals released by gradual and continuous decomposition of FYM in soil acted as nutrients for *T. harzianum*. The higher population of both *T. harzianum* and *T. viride* in mixed compost in comparison to individual one might be due to the cumulative effect of nutrient contents of both the composts. Mixed compost, *i.e.*, optimal and vermicompost (50: 50) which resulted in higher spore concentration (CFU / g) was considered for shelf life study of both the antagonists, *i.e.*, talc-based *T. harzianum* and commercial biofungicide *T. viride* (Table 1).

The colony forming units (CFUs) were significantly higher in OC + VC (50:50) + Th, followed by OC + VC (50:50) + Tv treatment (Table 2). When the mean population after different days of inoculation was considered, it was found to steadily increase and reach a peak after 30 days of incubation and thereafter it declined slowly. The population of both the antagonists was observed in higher trend after 120 days of incubation, however, growth of *T. harzianum* was significantly higher than that of *T. viride*. In the shelf life study in compost pit, pits filled with optimal compost + vermicompost (50: 50) + *T. harzianum* showed significantly higher population, followed by pits filled with optimal compost + vermicompost (50: 50) + *T. viride*. However, soil without compost inoculated with *T. harzianum* and *T. viride* separately reflected significantly less population of both the antagonists as compared to the growth on compost.

Table 3 indicates, the populations of *T. harzianum* and *T. viride* in all the treatments significantly increased for up to 60 days after incubation. Moreover, population of both the antagonists in compost manure mixture showed an

Table 1. Growth and multiplication of talc based formulation of *Trichoderma harzianum* and commercial biofungicide *Trichoderma viride* on different compost manure

Treatment	Population density (CFU x 10^7 g^{-1} of compost) of*Trichoderma* spp. in compost manure			
	0 day	15 days	30 days	45 days
T_1 = Optimal compost + *Trichoderma harzianum*	6.4 (7.81)[a]	51.00 (8.71)[c]	60.50 (8.78)[c]	71.00 (8.85)[c]
T_2 = Optimal compost + *Trichoderma viride*	6.2 (7.79)[b]	37.00 (8.57)[d]	38.75 (8.59)[f]	54.50 (8.74)[e]
T_3 = Vermicompost + *Trichoderma harzianum*	6.4 (7.84)[a]	49.75 (8.70)[c]	50.25 (8.70)[d]	63.75 (8.80)[d]
T_4 = Vermicompost + *Trichoderma viride*	6.2 (7.79)[b]	58.75 (8.77)[b]	69.00 (8.84)[b]	78.76 (8.90)[b]
T_5 = Optimal compost + Vermicompost (50:50) + *Trichoderma harzianum*	6.4 (7.81)[a]	74.00 (8.87)[a]	83.50 (8.92)[a]	85.25 (8.93)[a]
T_6 = Optimal compost + Vermicompost (50:50) + *Trichoderma viride*	(7.79)[b] (7.79)[b]	58.75 (8.77)[b]	69.00 (8.84)[b]	78.76 (8.90)[b]
T_7 = Talc based formulation of *Trichoderma harzianum* alone	6.4 (7.81)[a]	6.1 (7.79)[e]	5.9 (7.77)[g]	5.8 (7.76)[f]
T_8 = Commercial biofungicide *Trichoderma viride* alone	6.2 (7.79)[b]	5.8 (7.76)[f]	5.6 (7.75)[h]	5.3 7.72)[g]
SEM ±	0.014	0.010	0.003	0.004
$CD_{0.05}$	0.029	0.021	0.007	0.009

Means within columns separated by Duncan's multiple range test $P = 0.05$; figures in parentheses are angular transformed values

Table 2. Population dynamics of talc-based *T. harzianum* and commercial biofungicide *T. viride* in sterilized compost manure *in vitro*

Treatments	Population of *Trichoderma* spp. (x 10^7 CFU g^{-1})				
	0 day	30 days	60 days	90 days	120 days
T_1 = Optimal compost + Vermicompost (50: 50) + *T. harzianum* application	6.4 (7.81)[a]	78.00 (8.89)[a]	62.75 (8.80)[a]	24.75 (8.39)[a]	18.00 (8.25)[a]
T_2 = Optimal compost + Vermicompost (50: 50)+ *T. viride* application	6.2 (7.79)[b]	69.40 (8.84)[b]	59.00 (8.77)[b]	19.00 (8.28)[b]	12.00 (8.08)[b]
T_3 = Sterilized soil + *T. harzianum* application	6.4 (7.81)[a]	52.80 (8.72)[d]	39.00 (8.59)[c]	15.75 (8.20)[c]	8.00 (7.90)[c]
T_4 = Sterilized soil + *T. viride* application	6.2 (7.79)[b]	53.60 (8.73)[c]	37.00 (8.57)[d]	13.25 (8.12)[d]	4.00 (7.60)[d]
SEM±	0.012	0.002	0.005	0.006	0.025
$CD_{0.05}$	0.027	0.005	0.011	0.013	0.059

Means within columns separated by Duncan's multiple range test $P = 0.05$; figures in parentheses are angular transformed values

increasing trend for up to 90 days, while population of both the antagonists at 90 days declined when it was inoculated with sterilized soil. However, population at 120 days after incubation was higher than the initial population. The minimum population of *T. viride* as compared to *T. harzianum* was observed at 120 days after inoculation in all the treatments. This might be due to the available carbon source easily taken up by the antagonists, thereby growing rapidly and when stored food was exhausted, resulted in the decline of population. This finding is in agreement with Zaidi and Singh (2004), where the population of *T. harzianum* on compost declined slowly after 30 days. Earlier, Saju *et al*. (2002) reported that the population of *T. harzianum* in neemcake, coirpith and FYM declined slowly after 30 days. Ramakrishnan *et al.* (1994) and Jayarajan and Nakkeran (1996) observed that there was a slow and

Table 3. Population dynamics of talc based *T. harzianum* and commercial biofungicide *T. viride* in different compost manure in *vivo*

Treatments	Population of T. spp. (x 10^7 cfu g^{-1})				
	0 day	30 days	60 days	90 days	120 days
T_1 = Optimal Compost + Vermi Compost (50:50) + *T. harzianum* application	6.4 (7.81)	34.80 (8.54)[a]	78.00 (8.89)[a]	88.00 (8.94)[a]	66.00 (8.82)[a]
T_2 = Optimal Compost + Vermi Compost (50:50) + *T. viride* application	6.2 (7.79)	30.25 (8.48)[b]	69.40 (8.84)[b]	78.25 (8.89)[b]	52.00 (8.72)[b]
T_3 = Soil + *T. harzianum*	6.4 (7.81)	11.60 (8.06)[c]	15.25 (8.81)[c]	12.00 (8.08)[c]	7.00 (7.85)[c]
T_4 = Soil + *T. viride*	6.2 (7.79)	8.00 (7.90)[d]	11.75 (8.07)[d]	9.00 (7.95)[c]	5.00 (7.70)[c]
SEM±	0.13	0.012	0.007	0.010	0.018
$CD_{0.05}$	NS	0.027	0.015	0.024	0.042

Means within columns separated by Duncan's multiple range test $P = 0.05$; figures in parentheses are angular transformed values

gradual decline in the population of *T. harzianum* in talc-based formulation maintaining a significantly high level of antagonist population after 120 days of storage. This might be due to the production of some extra-cellular enzymes which might help in survival for long period. The spores of *T. harzianum* spp. in osmoticant medium contain higher level of trehalose, which help the organism to tolerate low osmotic potential and survive longer period in water stress condition (Das *et al.,* 2006).

ACKNOWLEDGMENT

The authors are very much thankful to the Head, Department of Plant Pathology Dr. A. K. Saikia, Assam Agricultural University, Jorhat, for providing necessary facilities to undertake these studies.

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