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Survivability of *Trichoderma harzianum* in talc powder

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The major constraints identified for erratic performance of *Trichoderma harzianum* in field were due to poor survival potential of *Trichoderma harzianumin* in different delivery system. The survival potential of *Trichoderma harzianumin* was tested by spraying spores of T4 isolate in talc powder ($CaCO_3$) and mixed vigorously. The viability of *Trichoderma* spp. (T4) was measured from that formulated product. It was found that up to 105 days, there was significant difference among the mean survivality of population. The interaction of viable spores along with day and concentration of the spores were also measured. The pooled data of analysis revealed that between the days there was significant difference of mean survivality, in different of concentration.

Key words: Talc powder, survivability, *Trichoderma harzianum*, population

INTRODUCTION

The genus *Trichoderma* is known to include several potentially promising hyperparasites/antibiotic producers that have been effectively used against a large number of soil borne plant pathogens (Chet *et al.* 1979; Lewis and Papavizas, 1991; Mahanty *et al.* 2000). Although several delivery systems have been devised (Conway *et al.* 1982; D'Souza *et al.* 2001) those can be conveniently applied to soil but are difficult to come by. To measure or check the survivality of the antagonist before field application is an important parameter. As long as the antagonist will survive at the rhizosphere, it will check the growth of pathogen at the rhizosphere. So, the desirable number of population or viable spores of antagonist are required as it is stored in different carrier media.

Karunanithi *et al.* (2001) have used different carrier materials, i.e. talc ($CaCO_3$), peat, lignite, gypsum ($CaSO_4$), kaolin and fly ash for screening the mass multiplication of *Trichoderma viride*. Talc ($CaCO_3$) and gypsum ($CaSO_4$) are significantly superior for maintaining the survival of *Trichoderma viride* even after 150 days (5 month) of storage. Sanyal *et al.* (2003) have processed / formulated viable population of five isolates of *Trichoderma harzianum* as prills using $CaCl_2$ and Ca-gluconate (Ca-G) as gellant for increasing shelf life of a biocontrol agent. The results have showed that survivability of *T. harzianum* was higher (60-77days) when Ca-G was used than when $CaCl_2$ (32-45 days) was used as gellant. Survivability of most isolates is higher when initial population of 10^9 was used than when it was $l0^6$/g of prills.

Due to the failure of efficient delivery system of *Trichoderma* spp. in the field a major constraint is identified due to erratic performance of the antagonist, (Papavizas *et al.* 1984). Having established the promise of some *T.harzianum* isolates on major betelvine pathogens like *Phytophthora* sp., *Athelia roljsii* Curzi and *Colletotrichum capsici* Syd. (Butler and Bisby) (D'Souza *et al.* 2001; Roy, 2001) at this laboratory, present investigation has been undertaken to study the survivability of *Trichoderma harzianum* in talc ($CaCO_3$) at different population levels.

MATERIALS AND METHODS

Spore suspension (10^{10}/ml) of T4 isolate of *T.harzianum* was prepared by using potato dextrose broth. The spore suspension was then

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sprayed on talc ($CaCo_3$) by a glass make atomizer. While spraying, the spore suspension of *T.harzianum* was mixed rigorously with talc.10g of *T.harzianum* inoculated talc was placed in 10 ml. distilled water and shaken vigorously.1ml of suspension was mixed with 9 ml of sterile water diluting it serially up to 10^{-5} dilution. 1ml of each dilution was then transferred aseptically into each sterile plate. Modified TSM poured on to replicated sterile Petri plates and rotated clock and anticlockwise for uniform distribution and then incubated at 28°C ± 1°C for 4 days. Colony growth was found after 4 days. The number of colonies formed in each dilution was recorded after 8 days of inoculation. The inoculated talc was kept in a plastic packet at normal room temperature and same colony counting was repeated after every 15 days intervals until colony formation was recorded nil at any dilution. The results obtained are subject to analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The result (Table 1; Fig. 1) revealed that the highest number of population was observed in day 0 at 10^{-10} concentration, with the increase of the day the population started to fall gradually. In day 105 at 10^{-10} concentration there was no population found to survive. Finally in day 120 except $10^{-5,}$ 10^{-6} concentration, there was no colony formation.

The pooled ANOVA data (Table 2) revealed that in different of concentration, between the days, there was significant difference for mean survivality value of *T.harzianum* at 1% level of significances.

The effect of day × concentration showed that there was highly significant interaction between day and population concentration of *T.harzianum* at 1% level of significances. With the increase of day and dilution the population was reduced (Table 2).

Another factor showed that indifferent of days, among the concentrations, there were significant mean survivality differences of *T.harzianum* (Table 1).

For the development of proper bioformulated product, proper delivery system or survivality in storage condition of bioagent (*Trichoderma* spp.), Calcium Carbonate (talc) was used as a substrate because it is a carbon containing substrate. Longa *et al*. (2008) showed *T. atriviriode* survived best in carbon source peptone, tryptone, nitrate, mannose, galactose, sucrose with highest biomass production. T4 isolate of *T.harzianum* was selected based on the superiority of it at dual plate technique against *Phytophthora parasitica* (Datta *et al*. 2011). The highest population was found in day 0 at 10^{-10} concentration. The pooled data of analysis revealed that between the days there was significant difference of mean survivality, indifferent of concentration. *T. koningii* biomass was formulated in sodium alginate suspension and dipping into $CaCl_2$ solution. cfu count was evaluated 4, 8, 12 weeks. After 12 weeks cfu was 3.7×10^4 (Anonymous, 1999). Among the concentrations there were significant differences for mean survivality values. Jin and Xixuan (2010) proposed 2% sucrose solution showed highest survival percentage in comparison to other sucrose solution having cfu 7.5×10^{10}/g. The interaction between concentration and day was also highly significant. *Trichoderma* survived well as a formulated product in calcium carbonate power. Karunanithi *et al.* (2001) showed talc ($CaCO_3$) and gypsum ($CaSO_4$) were superior for maintaining the survival of *T. viride* even after 150 days (5 months) of storage. Rice grain supplemented with $CaCO_3$ was used for delivery system (Rogerio *et al*. 2009). *Trichoderma* spp. was regularly recovered from the roots of spruce rhizosphere, added with calcium carbonate (Estivalet *et al.* 1990).

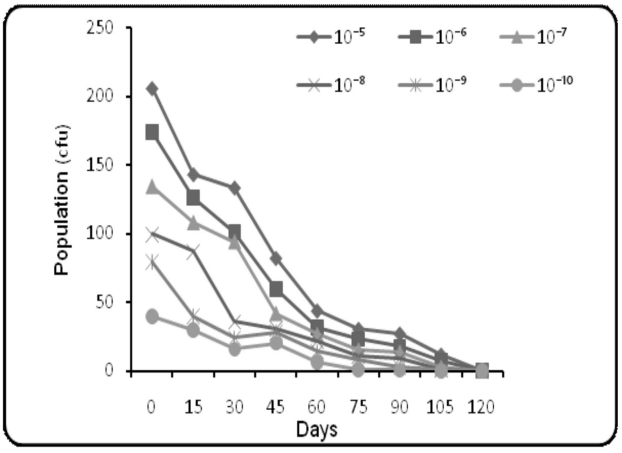

Fig. 1: Linear plot showing the effect of days and concentration for the survivality of *Trichoderma* sp. at formulated product

From the results it can be concluded that for better delivery system of *T.harzianum*, talc ($CaCO_3$) may be used for mass multiplication of the bioagent as the required population for effectiveness of the bio-agent for the control of plant diseases is maintained

Table 1: Survivality of *Trichoderma* spp.

Variable (Days)	cfu at different concentrations						
	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	Mean
0	206.00	174.33	134.67	99.67	79.33	40.00	122.33
	(14.37)	(13.22)	(11.63)	(10.01)	(8.93)	(6.76)	(10.75)
15	143.33	126.33	108.33	86.67	39.67	30.00	89.06
	(14.99)	(11.27)	(19.43)	(9.33)	(6.33)	(5.52)	(9.15)
30	133.67	101.33	94.33	36.00	24.00	16.00	67.56
	(11.58)	(10.09)	(9.74)	(6.04)	(4.95)	(4.06)	(7.74)
45	82.33	59.67	42.00	30.67	28.00	20.33	43.83
	(9.10)	(7.76)	(6.52)	(5.58)	(5.34)	(4.56)	(6.47)
60	44.00	32.00	26.67	21.67	14.00	6.67	24.17
	(6.67)	(5.76)	(5.20)	(4.69)	(3.79)	(2.64)	(4.78)
75	30.67	23.33	15.33	10.67	8.00	1.33	14.89
	(5.58)	(4.87)	(3.98)	(3.34)	(2.91)	(1.34)	(3.67)
90	27.33	18.00	13.67	8.67	2.33	0.67	11.78
	(5.27)	(4.30)	(3.75)	(3.03)	(1.68)	(1.05)	(3.18)
105	12.00	7.67	2.67	1.33	0.67	0.00	4.06
	(3.53)	(2.58)	(1.76)	(1.29)	(1.05)	(0.71)	(1.87)
120	0.33	0.33	0.00	0.00	0.00	0.00	0.11
	(0.88)	(0.88)	(0.71)	(0.71)	(0.71)	(0.71)	(0.76)
	Days (D)			Concentration (C)			D ? C
SEm (?)	0.607			0.496			1.488
CD (P=0.05)	1.702			1.391			4.172

Figure in parentheses are the transformed values.

Table 2 : Pooled ANOVA

Source	SS	df	MS	F	Sig.
Day	1649.01	8	206.13	3501.73	0.000
Day ? Concentration	128.17	40	3.20	54.44	0.000
Error (Day)	5.65	96	0.06		
Concentration	416.58	5	83.32	824.79	0.000
Error	1.21	12	0.10		

up to 105 days. However, most of the formulated bioagents available in the market is having expiry dates of 180-365 days. To increase the survivality of bioagent further study is needed with more numbers of highly effective bioagents having more survival periods in carrier agent.

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