Survival potential of *Trichoderma harzianum* in alginate prills

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ABSTRACT: Viable population of five isolates of *Trichoderma harzianum* were processed/ formulated as prills using $CaCl_2$ and Ca-gluconate (Ca-G) as gellant for increasing shelf life of a biocontrol agent. The results showed that survivability of *T. harzianum* was higher (60-77 days) when Ca-G was used than when $CaCl_2$ (32-45 days) was used as gellant. Survivability of most isolates was higher when initial population of 10^9 was used than when it was 10^6/g of prills.

KEY WORDS: Alginate prills, biocontrol, bioformulation, survival, *Trichoderma harzianum*

The genus *Trichoderma* is known to include several potentially promising hyperparasites/ antibiotic producers that have promise against a large number of soil borne plant pathogens (Chet *et al.*, 1979; Lewis & Papavizas, 1991; Mohanty *et al.*, 2000). Although several delivery systems have been devised (Conway *et al.*, 1982; D'Souza *et al.*, 2001) those that can be conveniently applied to soil are difficult to come by. Use of mycoherbicide alginate prills (Lewis & Papavizas, 1984) led to similar use of these formulations of biocontrol for plant pathogens where the agent is enmeshed in a polymer matrix and released shortly afterwards (Fravel *et al.*, 1985).

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, tailoring them into alginate prills was evaluated through use of two different gellants, at different population levels and their shelf life up to threshold was determined.

MATERIALS AND METHODS

Mass Production Technology of Prills

Five g of Na-alginate and 200 g of bentonite were added to a litre of sterile water and mixed in a blender for one min. To this mixture five and ten g of air dried biomass of *Trichoderma harzianum* was added and again blended for 30 seconds to generate two distinct population levels. These were then dripped through Pasteur pipettes having one mm orifice, into a solution of $0.25 M CaCl_2$ or $0.1 M Ca$-gluconate (Ca-G). Prills thus formed were harvested from the solution, rinsed in distilled water and spread in a single layer in trays lined with blotting paper. These prills, initially 3-4 mm in diameter, shrunk to 1-2 mm in diameter after air-drying in a laminar air flow but remained spherical. Each litre of alginate clay mixture produced ca. 110 g of air-dried formulated prills that was stored in screw capped jars under room conditions (15-30° C temperature and 55-90% RH).

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Determination of Viability of *Trichoderma* in Alginate Prills

Viability of *Trichoderma* in prills was quantified before exposing the organism to Ca-salt ($CaCl_2$/Ca-G) i.e., before prill formation, at 24 h (prills, 1st day of storage), and on every subsequent seven days after prill formation. The prills were disintegrated in a mixture of 8.7×10^{-2} M KH_2PO_4 and 3.0×10^{-2} M Na_2HPO_4, $7H_2O$. The mixture was then filtered and filtrate was taken as suspension of spores. Twelve prills were equivalent to 1 ml of suspended biomass. The population of *Trichoderma* was enumerated by dilution plating in modified TSM media {(Chet *et al.*, 1979) as modified by Sen (1998)} ($MgSO_4$,$7H_2O$= 0.20 gm; K_2HPO_4 = 0.90 gm; NH_4NO_3 = 1.00 g; KCl = 0.15 g; glucose/dextrose = 3.00 g; Redomil/mancozeb = 0.10 g; distilled water 1000 ml; agar-agar = 15 g. The medium was sterilised at 15 psi for 15 min. Dexon in the original formulation was replaced by methyl orange and captan. After autoclaving the following were added (mg L^{-1}): chloramphenicol-250; Methyl orange - 300; Brassicol (PCNB) 75% - 200; Rose Bengal - 150; Captan 50% WP - 10).

Two initial concentrations of spores (10^{-6} and 10^{-9}) were used. After plating, the plates were stored at 28±1°C for 3 days. After 3 days colony counts were recorded for each dilution. Three replications for each dilution were prepared. Two separate experiments were conducted to assess the survivability of *Trichoderma* sp. in the formulation using either $CaCl_2$ or Ca-G as gellant. The differences in colony character while using the two types of gellants were also recorded.

The entire test was conducted with five isolates of *T. harzianum, viz.*, T_c, T_1, T_2, T_3 and T_5. The survival data were subjected to linear regressions and time required for population to reach threshold level (ca 10^6 cfu/g prill)

RESULTS AND DISCUSSION

Population of *T. harzianum* showed a nearly continuous deceleration as a function of time, the deceleration gaining momentum after about 35 days. The pattern was uniquely uniform for all the isolates, generally irrespective of initial population or the type of gellant used (Table 1-4). However, this deceleration was almost linear from 2 weeks onwards for isolate T_3 while it was slow for remaining isolates for first 5 weeks and very rapid thereafter, when $CaCl_2$ was used as gellant at high population of *Trichoderma* (Table 1). With lower initial population a slow deceleration was observed up to 35 days for all the isolates. Subsequently such fall in population was rapid for isolate T_1, moderate in T_5 and T_2 and uniformly slow up to 50 days for T_c and T_3 (Table 2). Ca-G gellant produced prills in which the propagules survived longer than in those produced in $CaCl_2$. Population declined slowly up to 50 days and then rapidly up to 63 days for T_c, moderately for T_1 and T_5 and slowly for T_2 and then there was a rapid deceleration as a function of time

Table 1. Survival of *T. harzianum* in alginate prills at room temperature as a function of time using $CaCl_2$ as a gellant with high initial population

Isolate and initial conc. of spores	Cfu*per g of soil as a function of time					Regression Equation	R^2
	1st day	14 days	28 days	42 days	56 days		
T_c (39.4x10^9)	6.9x10^9	1.8x10^9	9.5x10^7	2.37x10^6	5.67x10^4	Y= 753.20 - 165.1x	0.90
T_1 (24.5x10^9)	4.5x10^9	1.2x10^9	8.3x10^7	1.49x10^6	3.37x10^4	Y= 519.98 - 111.64 x	0.94
T_5 (33.2x10^9)	5.2x10^9	1.5x10^9	9.9x10^7	1.68x10^6	4.08x10^4	Y= 552.04 - 120.02 x	0.88
T_2 (26.9x10^9)	6.9x10^9	2.2x10^9	8.3x10^7	2.36x10^6	2.23x10^4	Y= 820.48 - 176.14 x	0.96
T_3 (37.3x10^9)	6.3x10^9	3.7x10^9	7.7x10^7	1.86x10^6	5.35x10^4	Y= 861.02 - 174.56x	0.95

Table 2. **Survival of *T. harzianum* in alginate prills at room temperature as a function of time using $CaCl_2$ *as a gellant with low initial population**

Isolate and initial conc. of spores	Cfu*per g of soil as a function of time					Regression Equation	R^2
	1st day	14 days	28 days	42 days	56 days		
T_c (8.42×10^7)	2.45×10^7	1.19×10^7	3.7×10^6	8.8×10^5	4.92×10^4	$Y = 2379.1 - 343.76x$	0.89
T_1 (6.57×10^7)	1.75×10^7	9.70×10^6	2.9×10^6	$1.0 \times 10_5$	8.9×10^4	$Y = 1742.2 - 252.02x$	0.92
T_5 (7.28×10^7)	1.93×10^7	8.70×10^6	2.3×10^6	1.22×10^5	7.9×10^4	$Y = 184.88 - 272.83x$	0.87
T_2 (6.17×10^7)	1.64×10^7	9.20×10^6	2.3×10^6	2.1×10^5	9.0×10^4	$Y = 1650.2 - 239.26x$	0.91
T_3 (8.31×10^7)	2.59×10^7	1.22×10^7	4.2×10^6	7.9×10^5	8.9×10^4	$Y = 2485.8 - 358.01x$	0.89

* Cfu on TSM modified medium

Table 3. **Survival of *T. harzianum* in alginate prills at room temperature as a function of time using Ca- gluconate as a gellant with high initial population**

Isolate and initial conc. of spores	Cfu*per g of soil as a function of time							Regression Equation	R^2
	1^{st}day	14days	28days	42days	56days	70days	84days		
T_c 5.27×10^{10}	2.03×10^{10}	1.49×10^{10}	6.9×10^9	1.0×10^9	1.4×10^8	7.5×10^6	1.37×10^5	$Y = 232.84 - 30.839x$	0.98
T_1 6.78×10^{10}	2.23×10^{10}	1.66×10^{10}	9.3×10^9	2.4×10^9	3.5×10^8	7.02×10^6	1.36×10^5	$Y = 263.30 - 33.35x$	0.99
T_5 4.99×10^{10}	1.92×10^{10}	1.52×10^{10}	7.2×10^9	1.9×10^9	3.04×10^8	5.96×10^6	8.16×10^5	$Y = 223.37 - 28.301x$	0.99
T_2 6.23×10^{10}	2.24×10^{10}	1.49×10^{10}	8.7×10^9	2.2×10^9	5.79×10^8	6.8×10^6	1.31×10^5	$Y = 245.53 - 31.415x$	0.99
T_3 5.42×10^{10}	2.15×10^9	1.35×10^{10}	7.2×10^9	4.5×10^9	3.34×10^8	5.42×10^6	1.11×10^5	$Y = 233.6 - 28.783x$	0.98

* Cfu on TSM modified medium

in all the isolates (Table 3). With low population using Ca-G, a uniform rate of deceleration in population occurred for all isolates up to 63 days. Subsequently deceleration was rapid in T_1, moderate in T_5, T_2 and T_3 and slow for T_c (Table 4). These results showed that Ca-G probably is a more meaningful gellant and isolates of *Trichoderma* hardly differ in their ability to survive as a function of time.

It is known that *Trichoderma*, a good saprophyte, acts as hyperperasite at relatively high populations and the threshold has been fixed at around 1×10^6 cfu/g soil (Adams, 1990; Baker & Dickman, 1993). Survival of the isolates in the prills as a function of time was plotted as a function of time in the combinations tested with population transformed to log. Following linear regression, the time required for population to reach the threshold level (ca x 10^6 cfu/g prill) was determined (Table 5).

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Table 4. Survival of *T. harzianum* in alginate prills at room temperature as a function of time using Ca-gluconate as a gellant with low initial population

Isolate and initial conc. of spores	Cfu*per g of soil as a function of time						Regression Equation	R^2
	1^{st}day	14days	28days	42days	56days	70days		
T_c (7.25x 10^7)	3.95x10^7	3.05x10^7	2.19x10^7	1.18x10^6	5.3x10^6	8.6x10^4	Y=424.83-40.852x	0.99
T_1 (8.46x 10^7)	3.95x10^7	3.55x10^7	2.57x10^7	1.45x10^7	6.3x10^6	9.5x10^4	Y=492.88-47.588x	0.99
T_5 (7.97x10^7)	3.95x10^7	3.02x10^7	2.24x10^7	1.22x10^7	6.2x10^6	9.2x10^4	Y=484.65-48.256x	0.90
T_2 (8.03x 10^7)	3.95x10^7	3.12x10^7	2.31x10^7	1.35x10^7	5.2x10^6	7.3x10^4	Y=422.37-40.234x	0.99
T_3 (8.29x10^7)	4.33x10^7	3.42x10^7	2.62x10^7	1.51x10^7	6.4x10^6	9.3x10^4	Y=480.57-46.26x	0.99

* Cfu on TSM modified medium

Table 5. Survival time* of isolates of *T. harzianum* in alginate prills upto threshold population

Isolates	$CaCl_2$ gellant		Ca gluconate gellant	
	HIP	LIP	HIP	LIP
Tc	45	37	77	66
T_1	44	32	77	60
T_5	44	32	75	61
T_2	45	32	77	61
T_3	45	39	75	61

HIP = High initial population; LIP = Low initial population

*Data presented are time in days required to bring down the cfu/g to $1x 10^6$

All linear regressions showed highly significant correlation as indicated by their high R^2 values (Table 1-4). But for a few exceptions which may be artifacts, the time required for reduction of population to threshold level does not vary much as function of the isolate. However, survival is far better in Ca-G formulated prills than $CaCl_2$ formulated ones. Halving the initial population does not affect shelf life concomitantly.

These results showed that this kind of prill, to be effective, need to be used immediately after preparation; Ca-G is a better gellant than $CaCl_2$; and it will be ideal to introduce some slowly utilisable nutrient source to expand the shelf life of *Trichoderma* in alginate prills. Work on the later aspect is in progress.

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