

Production of Mycofungicidal Pellets from *Chaetomium globosum*

Kasem Soyong* and Denise Witteman ☆

Abstract

Mycofungicidal pellets were produced from *Chaetomium globosum* by using spore suspension (4×10^6 spores/ml.) mixed to 1% sodium alginate and 10% hydrous aluminum silicate for one litre, then agitated through an electrical blender. The pH of such mixture was 6.8. The mixture was dripped into either a solution of 0.1 M calcium gluconate or 0.25 M calcium chloride for immediately formed pellets. The alginate pellets were then air dried for 12 hours. The

average diameter of dried pellets were approximately 3 mm. Survival tests at 24 hr and 4, 8, and 12 weeks revealed that the pellets with calcium chloride and calcium gluconate are not significantly different of survival during storage at room temperature. However, the percentage of survival declined with time during storage.

Keywords : mycofungicidal pellets, *Chaetomium globosum*

Research interests in biological control has been being increased using specific microantagonist to suppress many plant pathogens. Because of chemical control of diseases, though effective, is often subjected to misuse and abuse which often leads to associated problems such as fungicide residues, the destruction of non-target beneficial microorganisms and environmental pollution. Biological control of plant pathogens is a sustainable control method and is a component of integrated disease management. It does not promise total eradication of the problems encountered but reduces the pathogen population to an acceptable level with no detriment to the environment. There are many methods for for-

mulating and producing mycofungicides to deliver of biological control agents, e.g. application as seed treatments (Soyong and Quimio, 1989; Cullen *et al.*, 1984; Kommedahl and Mew, 1975) in organic matter (Wells *et al.*, 1972). Using sodium alginate for corporation of microbial herbicides have potential for use with biological control agent (Walker and Connick, 1983). The production of biodegradable pellets of relatively uniform size which are convenient for storage and survival (Fravel *et al.*, 1985). The aims of this study were to determine a biological control agent would survive in alginate pellets and to evaluate the storage duration at room conditions.

* Department of Plant Pest Management, Faculty of Agricultural Technology, King Mongkut's Institute of Technology, Bangkok 10520

☆ Faculty of Environmental Science, Prof. H.C. van Hall Institute, Hereweg 99 9721 AA, Groningen, The Netherlands

Materials and Methods

The mixture of 10 g sodium alginate and 100 g hydrous aluminum silicate was added to one litre of sterilized distilled water and comminuted in an electrical blender for 1 min. One hundred millilitres spore suspension of *Chaetomium globosum* after grown on Potato Dextrose Agar (PDA) for 4 weeks which counted the initial concentration was 4×10^6 spores/ml. The spore suspensions were amended to the alginate mixture which consisting of K_2HOP_4 -buffered 0.1%. The alginate-propagule mixture was stirred continuously when dripped through pipettes into a solution of 0.1 M calcium gluconate ($C_{12}H_{22}CaO_{14}$) or 0.25 M $CaCl_2$. The alginate pellets were dried overnight in a laminar air flow, and stored under room conditions in screw-capped jars. Propagule

viability and survival were evaluated using dilution plating. Dilutions of propagules were made onto PDA and repeated with four plates each at 10^{-1} , 10^{-2} , 10^{-3} and 10^{-4} dilutions. Colony forming units/ml was counted and percentage of survival was then formulated as follows:- counted colony forming units/initial colony forming units $\times 100$.

Results

Chaetomium globosum can survive in alginate pellets when using gelling agent either in Ca gluconate or $CaCl_2$. The viable of ascospores at 12 weeks was 81.65% and 90.30% for Ca gluconate and $CaCl_2$, respectively (Table 1). The percentage of propagule survival in alginate pellets at room conditions were

Table 1. Propagule viability in alginate pellets at room temperature of *Chaetomium globosum* used as biological control agent.

Gellant	Concentration of alginate pellets	Colony forming unit/ml.				Mean
		24 hr	4 wk	8 wk	12 wk	
Ca gluconate	10	125	108	85	75	98.25 ab [☆]
	10^{-1}	102	95	80	70	86.75 b
	10^{-2}	97	90	77	66	82.50 b
	10^{-3}	85	80	68	60	73.25 bc
	10^{-4}	79	75	62	54	67.50 c
	-		97.6	89.6	74.4	65.0
$CaCl_2$	10	130	127	105	90	113.00 a
	10^{-1}	126	115	92	79	103.00 a
	10^{-2}	102	98	81	68	87.25 b
	10^{-3}	90	86	74	62	78.00 b
	10^{-4}	81	79	63	58	70.25 bc
	-		105.8	101.0	83.0	71.4

[☆] Means of sixteen replications, within column followed by the same letter are not significantly different at the 5% level using DMRT.

Table 2. Percentage of propagule survival in alginate pellets at room temperature of *Chaetomium globosum* used as biological control agent.

Gellant	Concentration of alginate pellets	Propagule survival (%) [☆]			Mean
		4 wk	8 wk	12 wk	
Ca gluconate	10	86.4	68.0	60.8	71.73 a [★]
	10 ⁻¹	93.1	78.4	68.6	80.03 a
	10 ⁻²	92.7	79.3	68.0	80.00 a
	10 ⁻³	94.1	80.0	70.5	81.53 a
	10 ⁻⁴	94.9	78.4	68.3	80.53 a
	—	92.24	76.82	67.24	78.76
CaCl ₂	10	97.6	80.7	69.2	82.5 a
	10 ⁻¹	91.2	73.0	62.6	75.6 a
	10 ⁻²	96.0	79.4	66.6	80.66 a
	10 ⁻³	95.5	82.2	68.8	82.16 a
	10 ⁻⁴	97.5	77.7	72.5	82.56 a
	—	95.56	78.60	67.94	80.70

[☆] Propagule survival (%) = counted colony forming units / initial colony forming units × 100.

[★] Means of twelve replications, within column followed by the same letter are not significantly different at 5% level using DMRT.

not significantly different when used either Ca gluconate or CaCl₂ as gellants (Table 2). However, it was revealed that survival percentage declined with time during storage condition at room temperature, but the maximum survival time of *C. globosum* in alginate pellets was greater than 12 weeks (wk). The alginate pellets formed in round shape and the average size was 3 mm. in diameter (Figure 1).



Figure 1. Sodium alginate propagule pellets were formulated immediately when dripped to a solution of gellant (top) and after dried overnight (bottom).

Discussion

The possibility production of mycofungicidal pellets were demonstrated by these experiments. *C. globosum* would survive in alginate clay pellets gelled with either Ca gluconate or CaCl₂ remained viable for 12 weeks. The aglinate pellets formulated in round shape which could be functioned as a sources of living propagules. It was indicated by colony forming unit onto dilution plating. In this study, it showed propagule survival after 12 weeks of alginate pellets were not significantly different when using Ca gluconate or CaCl₂ as gellants that was not the same result with the work of Fravel *et al.* (1985). They stated that the survival of propagule alginate pellets after 12 weeks of all tested organisms like *Gliocladium virens*, *Penicillium oxalicum*, *Pseudomonas cepacia*, *Talaromyces flavus*, and *Trichoderma viride* encapsulated by using Ca gluconate as a gelling agent was significantly greater than when CaCl₂ was used. However, Fravel *et al.* (1983) pointed out that survival of alginate-propagule pellets for 12 weeks at room temperature should be sufficient time to make biocontrol formulations feasible for use. This study is the first report for using Ca gluconate and CaCl₂ as gellants for sodium alginate to enhance the survival of *C. globosum* used as mycofungicide. It is more feasible to formulate a biocontrol agent to facilitate for commercial production of mycofungicide, because it is not expensive and very versatile in size. However, Frave *et al.* (1985) stated that sodium alginate and CaCl₂ have been commonly used as food additives and nontoxic to nontarget organisms. It is a major challenge in plant pathology to develop new disease strategies using mycofungicide for controlling of plant pathogens.

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