LABORATORY REPORT



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Client:

Red Pod Inc. Boon Lim

Date: Project #:

July 24, 2009 09P1192 Barley

Purpose

Evaluate the composting material used in the biodegradation of one (1) material per ASTM D-6002 (germination) and by the plant growth test for toxicity per OECD 208 (summer barley evaluation).

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- A. Coated Hot Cup - 22.0 mil
- B. Blank Compost Material
- C. Standard Soil
- D. Summer Barley

Source: Red Pod Inc. Monterey Mushroom Inc. AMC, Inc./UIC Montana Seed and Grain

<u>Results</u> : The seed germination capacity for the one sample was above the recommended 90%.

The one (1) sample/compost material passed the OECD 208 (barley seed) toxicity test.

Discussion: The evaluation of the compost material used in the evaluation of the Coated Hot Cup samples were run per ASTM D-6002 Sec. 6.5 and OECD 208. Summer barley seed purchased from Montana Seed and Grain were used in the

# Section I - Barley Seed Germination

Dilutions were used from the supernatant that was collected from the wash of the composting material. Dry compost was washed with RO Water in a 0.0029 inch (200 mesh) USA Standard Testing Sieve. The RO water was poured over the compost while in the sieve. A blank control, a 25%, 50% and 75% dilution and a 100% concentrated supernatant were used for the test. Twenty seeds were placed on filter paper and 10 ml of each type of dilution was added. The samples were placed in a dark area at room temperature for four (4) days. After four (4) days, the samples were evaluated to determine the level of germination for each group. All dilutions had a germination capacity over the recommended 90%. Refer to Table III.

The seed germination was also recorded using the petri dish method. Twenty (20) seeds were put on moist filter paper in a petri dish and placed in a dark area for four (4) days. The percentage of seeds germinated were recorded. The average number of Summer Barley seeds germinated using the petri dish method was 97.5%. Refer to Table IV for a complete listing of values.

# Section II - Plant Growth

A 400 ml plastic beaker was used for each pot. Drainage holes were punctured at the bottom of each pot. This allows for air flow, drainage of excess water, and also water to wick up through the soil/compost material to the roots of the plants. The room temperature was controlled and kept constant around  $22^{\circ}$  C +/-  $10^{\circ}$  C. The humidity was also monitored for a constant reading of 70% +/- 25%. The plants were exposed to seventeen (17) hours of light and seven (7) hours of darkness. The testing period lasted for two (2) weeks.

Each compost sample from ASTM D 6868 was dried before the OECD 208 was started. The compost was then ground in a blender to eliminate large pieces. The replicates that required a compost/soil mixture were blended together for uniformity. The seeds were placed 0.5" below the top of the planting material.

In total, eighteen (18) flower pots per sample type were used (three replicates each of the six different mixtures) for the OECD 208 Test (summer barley seed) toxicity test. The various amounts and ratios of standard soil, blank compost, and the one (1) sample compost material are given on a volumetric basis and are listed in Table I, individual densities and test set-up summer barley seed which is attached. The volumetric densities were determined by dividing the dry weight (grams) of each compost sample type by 150 ml. These values represent one pot of each sample type. The remaining pots had the same amount of material in them. The standard soil, which was used was a potting soil produced by United Industries Corporation in St. Louis, Mo and it contained 40-50% peat.

The amount of barley seeds that germinated during the OECD 208 were also counted. One and a half (1.5) grams of barley seeds were placed into each compost sample type for observation. This is approximately 50 seeds (1.5 g  $\sim$  50 Barley Seeds). Fifty seeds were planted in each pot of each replicate. The number of seeds that were going to be used for the germination study (50 seeds) were counted before they were planted. The number of germinated barley seeds (sprouted) were then counted at the end of the test period. The percent germination from each pot was recorded. Refer to Table V.

The test was stopped after 14 days. Table II shows the fresh weight and dry weight yields (of above ground plant parts) for each test series along with their standard deviations. Comparison is based on the dry weight yields and not the fresh weight due to weight differences caused by watering/evaporation and compost mixtures. Table V represents the average number of barley seeds that germinated in each pot for each sample type.

The results in Figure I shows the Coated Hot Cup sample/compost material had no significant reduction on the plant dry yield of summer barley compared to the blank compost. Visual observations during the test and at the end showed healthy summer barley plants in all test series.

In conclusion, there was no reduction in the germination and growth of summer barley in compost obtained at the end of the ASTM D-6868 aerobic composting test of the Cup sample material. This means the sample material had no residual effects which negatively influenced the summer barley seed plant growth.

Client: Red Pod Inc. **OECD 208** Barley Sample A - Coated Hot Cup - 22.0 mil

Table I: Individual densities and amounts per pot for standard soil, blank compost and Coated Hot Cup Compost

Treatment	Soil or Compost (ml/pot)	Volumeric Density (kg/L)	Soil or Compost (g/pot)
Blank Compost	150	0.417	62.50
Standard Soil	150	0.299	44.80
Coated Hot Cup Compost	150	0.621	93.20

Test Set-Up Summer Barley Seed

Treatment	Soil or Compost (ml/pot)	Volumeric Density (kg/L)	Soil or Compost (g/pot)
3-Blank Compost	150	0.417	62.50
3-Standard Soil	150	0.299	44.80
3-Blank Compost/Soil (1:1)	150	0.358	53.65
3-Blank Compost/Soil (1:3)	150	0.328	49.23
3-Coated Hot Cup Compost/Soil (1:1)	150	0.460	69.00
3-Coated Hot Cup Compost/Soil (1:3)	150	0.379	56.90

### Table II: Fresh and Dry weight of Barley for each test series: Absolute and as percentage relative to Blank Compost.

	Fresh Weight Yield			
Test Series	(grams)		(% relative to Blank Compost)	
	AVG.*	STD**	AVG.*	STD**
3-Blank Compost	4.73	0.26	-	-
3-Standard Soil	4.79	0.72	101.27	0.15
3-Blank Compost/Soil (1:1)	5.20	0.56	109.94	0.12
3-Blank Compost/Soil (1:3)	6.06	0.52	128.12	0.11
3-Coated Hot Cup Compost/Soil (1:1)	5.94	0.61	125.58	0.13
3-Coated Hot Cup Compost/Soil (1:3)	6.88	0.67	145.45	0.14

	Dry Weight Yield				
Test Series	(grams)		(% relative to Blank Compost)		
	AVG.*	STD**	AVG.*	STD**	
3-Blank Compost	0.44	0.02			
3-Standard Soil	0.49	0.02	111.36	0.04	
3-Blank Compost/Soil (1:1)	0.49	0.04	111.36	0.09	
3-Blank Compost/Soil (1:3)	0.56	0.03	127.27	0.07	
3-Coated Hot Cup Compost/Soil (1:1)	0.53	0.03	120.45	0.07	
3-Coated Hot Cup Compost/Soil (1:3)	0.56	0.02	127.27	0.04	

\*AVG = Average \*\* STD = Standard Deviation

Client: Red Pod Inc. OECD 208 Barley Sample A - Coated Hot Cup - 22.0 mil

# Table III : ASTM D 6002 Germination Values of Barley Seeds Using 50 Seeds Per Container

Treatment (%) and Replicate #	# Germinated	% Germination	Mean % Germination	Mean % Difference in Germination from Control
BK Control - 1	49	98		
BK Control - 2	49	98	98	-
25 - 1	49	98		
25 - 2	48	96	97	1.0
50 - 1	48	96		
50 - 2	48	96	96	2.0
75 - 1	48	96		
75 - 2	47	94	95	3.0
100 - 1	47	94		
100 - 2	46	92	93	5.0

### Table IV : ASTM D 6002 Germination Values of Barley Seeds Using 20 seeds Per Petri Dish

Petri Dish	# of Barley Seeds	# of Seeds Germinated	Germination %
1	20	20	100.0
2	20	19	95.0

## Table V : OECD 208 Average Germination Values of Barley Seeds (1.5 grams ~ 50 seeds per Sample Pot) Per Sample Type

94
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94
94
96
96
98

