ASTM E1963 - Terrestrial Plant Toxicity Study - 1115240213B

To: Nassimi

Report Number: 1115240213B

Date: April 11, 2023

From: Thomas Poth - Eden Research Laboratory

Regarding: 23 days test of Nassimi Sample ERL# 3734

Eden Research Laboratory

2412 Princeton NE, Suite E
Albuquerque, NM 87107
T 505.508.3994
thomas.poth@edenresearchlab.com

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RESULTS

Following 27.5 months of biodegradation as per ASTM D5511, no mutations are apparent using the residual inoculum of exposed Nassimi Sample ERL# 3734 when compared to the inoculum alone.

METHOD

ASTM method E1963 measures the effect of substances on plant growth. The method details several measurements to determine the toxicity of test substances. Depending on the nature of the test substance the method allows for mixing of the test substance directly into the seedling mix or extraction of the substance and addition of this to the mix. The test stipulates that testing be done under controlled illumination and consistent temperature and humidity.

Evaluation of seedling emergence, epicotyl and hypocotyl length as well as epicotyl weight. Among the many species mentioned in the method bean, corn and pea give very good results. Boron positive control is employed to compare results from run to run.

Seeds are screened in advance to achieve a uniform size, then stored in a cool, dry place. Seeds are then planted, 2-4 per cell, depending on size to a depth of 1.5 to 2 times the seed diameter in a mixture of potting soil and perlite. The test is run in duplicate with from 5 to 20 seeds per duplicate. Flats are then placed under fluorescent lights with an illumination of 100-200 uM m-1 s-1 for 12 to 16 hours per day. Two weeks later growth is terminated and data is collected. Temperature and humidity are kept within narrow limits and monitored constantly.

DATA

Four seed groupings are germinated. They are as follows:

GROUPING	DESCRIPTION
POSITIVE CONTROL	General Soil & 20mM Boric Acid
SAMPLE	3 Parts General Soil & 2 Parts Sample Exposed Inoculum
INOCULUM	3 Parts General Soil & 2 Parts Inoculum

POSITIVE (in cm and g)

BEAN													
EP LENGTH	5.0	2.5	4.5	3.0	3.0	2.0	3.5	2.5	2.0	2.5	3.0	3.5	3.0
HY LENGTH	0.7	0.8	0.7	0.8	0.5	0.8	0.8	0.7	0.6	0.6	0.5	0.7	0.5
EP WEIGHT	0.8	0.9	0.7	0.9	0.7	0.8	0.8	0.8	0.6	0.6	0.6	0.7	0.6
CORN													
EP LENGTH	4.5	4.5	7.5	6.0	5.5	6.5	6.5	8.5	6.0	9.0	4.5	5.0	9.5
HY LENGTH	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.4	0.2	0.2	0.4
EP WEIGHT	0.9	0.9	0.9	1.1	1.1	1.0	0.9	1.0	1.0	1.2	1.1	1.0	1.3
WHEAT													
EP LENGTH	17.5	16.0	19.0	15.0	15.0	19.0	17.0	20.5	20.0	19.0	20.0	15.0	15.0
HY LENGTH	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
EP WEIGHT	0.3	0.2	0.3	0.1	0.1	0.2	0.1	0.3	0.2	0.2	0.2	0.1	0.1

BEAN													
EP LENGTH	2.5	2.5	2.5	2.5	1.5	2.0							
HY LENGTH	0.8	0.7	0.9	0.9	0.7	0.7							
EP WEIGHT	0.8	0.7	0.9	0.9	0.7	0.7							

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CORN													
EP LENGTH	5.5	8.5	6.5	6.0	6.5	5.5	8.0	6.0	7.5	4.5	4.0	3.5	
HY LENGTH	0.2	0.3	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.1	0.2	0.1	
EP WEIGHT	1.2	1.1	1.0	1.0	0.9	0.9	1.0	1.2	1.0	1.2	0.9	1.0	
WHEAT													
EP LENGTH	16.0	16.0	18.0	20.5	11.5	17.0	16.0	19.0	19.0	17.5			
HY LENGTH	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1			
EP WEIGHT	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.1			

INOCULUM (in cm and g)

BEAN													
EP LENGTH	9.0	19.0	19.0	16.5	14.0	19.0	16.5	19.0	12.0	15.5	18.0	15.5	16.0
HY LENGTH	1.0	1.0	1.0	1.0	0.7	1.4	0.9	1.2	0.6	0.6	1.0	0.9	0.9
EP WEIGHT	1.3	1.4	1.3	1.4	1.0	2.0	1.3	1.6	0.9	0.8	1.3	1.1	1.4
CORN													
EP LENGTH	15.5	18.0	28.0	13.5	17.0	17.0	8.0	19.5	11.0	24.0	16.0	11.5	16.0
HY LENGTH	0.4	0.6	90.0	0.5	0.6	0.6	0.2	0.8	0.3	0.7	0.4	0.4	0.5
EP WEIGHT	1.8	1.9	2.6	1.6	1.8	2.2	1.0	2.4	1.0	2.6	1.6	1.6	2.0
WHEAT													
EP LENGTH	24.0	24.5	24.0	26.0	26.0	27.0	24.0	27.0	27.0	23.0	24.0	16.5	26.0
HY LENGTH	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.0	0.1
EP WEIGHT	0.5	0.3	0.4	0.4	0.3	0.5	0.4	0.6	0.5	0.5	0.3	0.1	0.3

BEAN												
EP LENGTH	14.0	15.5	14.0	14.0	16.0	17.0	13.0	14.0	20.5	10.0	11.0	16.0
HY LENGTH	0.9	0.9	1.3	1.0	0.9	1.1	0.8	0.9	1.1	1.0	0.6	1.2
EP WEIGHT	1.3	1.4	1.5	1.4	1.1	1.5	1.1	1.2	1.4	1.3	0.8	1.6
CORN												
EP LENGTH	14.0	21.0	21.0	17.0	24.5	17.5	14.0	21.5	23.0	20.0	20.5	15.5
HY LENGTH	0.4	0.8	0.7	0.5	0.9	0.5	0.5	0.8	0.7	1.8	0.6	0.4
EP WEIGHT	1.8	1.9	2.2	1.8	2.3	2.6	1.5	2.5	2.3	2.0	2.2	1.9
WHEAT												
EP LENGTH	24.0	23.0	24.0	25.0	25.0	25.0	28.0	26.0	25.0	15.0		
HY LENGTH	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	1.0		
EP WEIGHT	0.2	0.3	0.2	0.3	0.4	0.5	0.4	0.4	0.2	1.1		

NASSIMI (3734) - SAMPLE (in cm and g)

BEAN													
EP LENGTH	21.0	22.0	23.0	18.0	19.5	18.0	19.0	17.0	19.0	20.0	18.0	13.0	22.0
HY LENGTH	3.0	2.5	3.5	2.5	2.0	2.5	2.0	2.5	3.0	3.5	4.0	2.0	2.5
EP WEIGHT	5.5	3.0	5.5	3.0	3.0	5.5	4.0	5.0	6.5	6.5	6.0	2.5	4.0
CORN													
EP LENGTH	4.0	3.5	3.0	7.0	18.0	24.0	14.0	30.0	40.0	17.0	40.0	20.0	40.0
HY LENGTH	2.5	1.8	1.9	2.0	2.3	2.5	2.2	2.3	2.0	1.8	2.5	1.0	1.5
EP WEIGHT	1.8	1.6	1.8	1.7	1.0	2.5	2.0	1.5	2.8	1.5	4.5	2.5	3.5
WHEAT													
EP LENGTH	35.0	30.0	35.0	35.0	3.0	35.0	30.0	26.0	30.0	30.0	35.0	30.0	35.0
HY LENGTH	1.2	0.5	1.4	1.1	0.5	1.8	1.2	0.8	0.5	1.0	0.5	1.2	0.5
EP WEIGHT	2.0	1.6	1.0	2.2	1.6	1.8	2.0	2.0	2.0	1.0	1.5	1.8	1.9

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BEAN													
EP LENGTH	20.0	17.0	14.0	17.0	20.0	16.0	11.0	16.0	14.0	16.5	15.0	14.0	
HY LENGTH	3.0	2.5	1.5	2.5	3.0	3.5	1.0	3.0	3.0	2.5	2.5	1.5	
EP WEIGHT	1.0	5.5	2.0	4.5	7.5	6.0	2.5	5.0	8.0	4.5	4.5	4.0	
CORN													
EP LENGTH	45.0	25.0	25.5	23.5	20.0	17.0	30.0	35.0	30.0				
HY LENGTH	2.2	2.3	1.0	0.5	3.0	2.5	1.8	2.2	1.7				
EP WEIGHT	3.0	2.2	3.0	2.5	6.5	5.0	2.1	2.0	1.8				
WHEAT													
EP LENGTH	35.0	30.0	35.0	35.0	30.0	35.0	30.0						
HY LENGTH	1.8	0.5	1.8	1.1	0.5	1.6	0.5						
EP WEIGHT	1.5	2.0	2.5	2.3	2.0	1.8	1.6						

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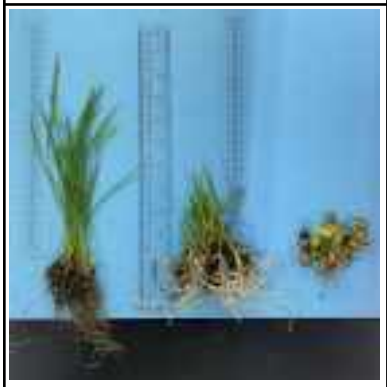
POSITIVE	Verde Valle 3533	INOCULUM
		

		AVERAGE (cm)	% GERMINATION	AVERAGE (g)
POSITIVE				
BEAN				
	EP LENGTH	2.8	76.0	
	HY LENGTH	0.69		
	EP WEIGHT			0.74
CORN				
	EP LENGTH	6.4	92.0	
	HY LENGTH	0.22		
	EP WEIGHT			1.03
WHEAT				
	EP LENGTH	17.3	92.0	
	HY LENGTH	0.07		
	EP WEIGHT			0.16
INOCULUM				
BEAN				
	EP LENGTH	15.4	100.0	
	HY LENGTH	0.95		
	EP WEIGHT			1.29
CORN				
	EP LENGTH	17.8	100.0	
	HY LENGTH	4.17		
	EP WEIGHT			1.96
WHEAT				
	EP LENGTH	24.3	92.0	

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	AVERAGE (cm)	% GERMINATION	AVERAGE (g)
HY LENGTH	0.16		
EP WEIGHT			0.40
SAMPLE 3734			
BEAN			
EP LENGTH	17.60	100.00	
HY LENGTH	2.60		
EP WEIGHT			4.60
CORN			
EP LENGTH	23.25	88.00	
HY LENGTH	1.98		
EP WEIGHT			2.58
WHEAT			
EP LENGTH	30.95	80.00	
HY LENGTH	1.00		
EP WEIGHT			1.81

CONCLUSION

Upon consideration of the data, it becomes apparent that there is no effect of biodegraded products of the Nassimi Sample ERL# 3734 when subject to the conditions of the ASTM D5511-18. The bean are a good indicator, since they are very sensitive to soil conditions, that the inoculum is still “hot” from high levels of nutrients out of balance with required distribution.

The Nassimi Sample shows no inhibitory effect from the inoculum after exposure to the plastic and its biodegradation products. Considering noticeable mutations in the positive control it is worth noting there are no indications of mutation in size or shape in the Verde Valle sample.

Thomas Poth
Laboratory Director
Eden Research Laboratory