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"GROWTH OF PLANT TISSUE CULTURES IN SIMULATED LUNAR SOIL - IMPLICATIONS FOR A LUNAR BASE CELSS".

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ABSTRACT

Experiments to determine whether plant tissue cultures can be grown in the presence of simulated lunar soil (SLS) and the effect of simulated lunar soil on growth and morphogenesis of such cultures, germination of seeds and development of seedlings were carried out in this laboratory.

Studies were scaled down to minimum and optimum usage of the small amounts of SLS which was available.

Our preliminary results on seed germination and seedling growth of Rice and calli growth of winged bean and soybean indicate that there is no toxicity or inhibition of SLS at all, even though SLS contains high amounts of Aluminium compounds compared to earth soil. Also SLS can be used as a support medium with supplements of certain specific major and micro elements.

GROWTH OF PLANT TISSUE CULTURES IN SIMULATED LUNAR SOIL - IMPLICATIONS FOR A LUNAR BASE CONTROLLED ECOLOGICAL LIFE SUPPORT SYSTEM.

This report will cover the period from February 1, 1987 to July 31, 1987.

1.0 INTRODUCTION.

Lunar based agriculture can perform a vital role in providing food, landscape and other physiological aspects for future manned missions to the moon and manned interplanetary exploration. Studies on lunar based agriculture are limited due to unavailability of lunar soil and Simulated Lunar Soil (SLS) is substituted in creating the lunar environment on earth, for such studies.

The SLS used in the experiments carried out in this laboratory were supplied by Dr. Don Henninger of Johnson Space Center, NASA, Houston. A total of 20 grams of highland SLS #3, which had the chemical composition of the Highland basaltic soil (table 1) of moon were obtained. Soil was greyish in color and had the appearence of a coarse powder.

Studies carried out to determine the effect of SLS on germination of seeds, growth and development of seedlings and growth and morphogenesis of plant tissue cultures are listed below in subsequent chapters.

Table 1.
Composition of Highland basaltic soil.

OXIDE	WEIGHT %
MgO	6.1
FeO	4.6
TiO	0.4
Cr O	0.1
Al O	27.4
CaO	15.6
Na O	0.4
K O	0.1
SiO	45.3

2.0. Seed germination in the presence of simulated lunar soil (SLS).

2.1. Rice seed germination; Variety BG379-2:

Seeds of the variety BG 379-2 obtained from Sri Lanka were used in this experiment. Seeds were treated with 100 mg of simulated lunar soil, sprinkled on them and the control was subjected to the similar conditions except for the presence of the SLS (Figure 1). Each treatment containing 20 randomly selected seeds was replicated two times.

Germination percentage of 85% was observed in both the control and treatment. Seedling heights were measured 2 weeks into germination (Figure 2) and are given in table 2.

Table 2.
Length of rice seedlings (in centimeters) of variety BG379-2 after 2 weeks;

CONTROL		TREATMENT	
Replicate 1	Replicate 2	Replicate 1	Replicate 2
2.9	2.6	3.6	4.5
2.7	1.6	4.0	3.3
2.0	2.0	3.4	2.7
2.8	3.0	3.1	4.0
2.9	1.5	3.0	3.5
2.0	2.4	2.4	2.9
2.5	2.7	4.0	3.9
2.6	3.0	4.4	3.4
3.0	2.2	2.5	3.6
3.3	2.5	3.6	4.0
2.7	2.3	3.0	3.4
2.5	1.5	4.3	3.2
1.8	2.5	2.9	3.6
0.6	1.6	3.0	2.0
2.3	2.1	1.5	1.1
3.2	2.2	2.7	0.1
2.2	2.0	0.1	0.2

Statistical analysis of varience (ANOVA) of data of table 2 is given in APPENDIX 1. Comparison of the treatment and the control indicated a significant increase of the seedling length of the rice seedlings grown in the presence of SLS.

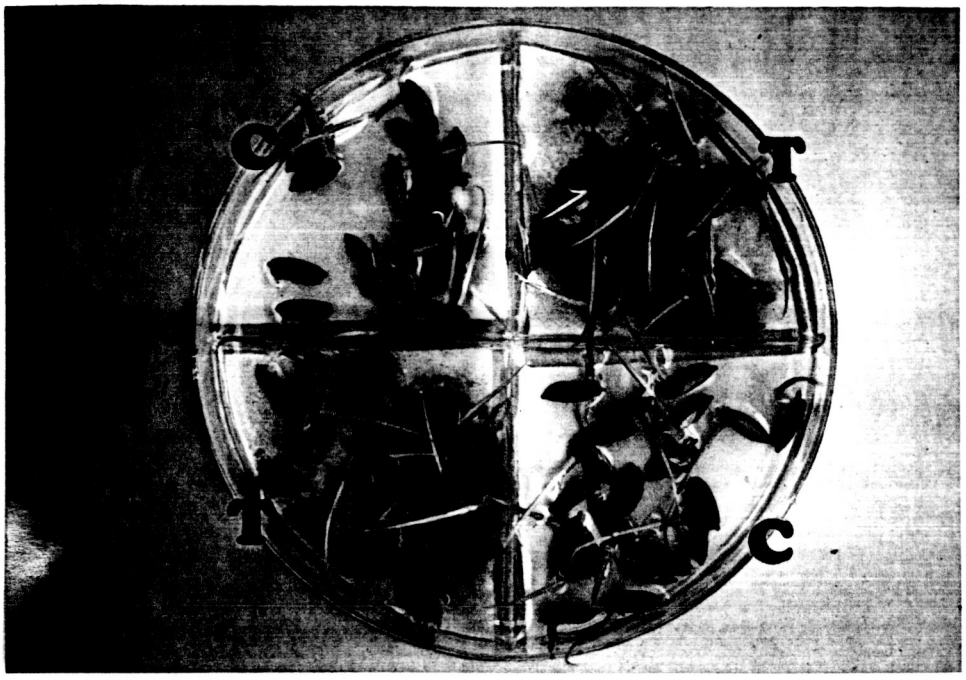


Fig: 1. Rice seeds germinating on petri dish. T = seeds germinating in the presence of SLS, C = control, seeds germinating with no SLS.

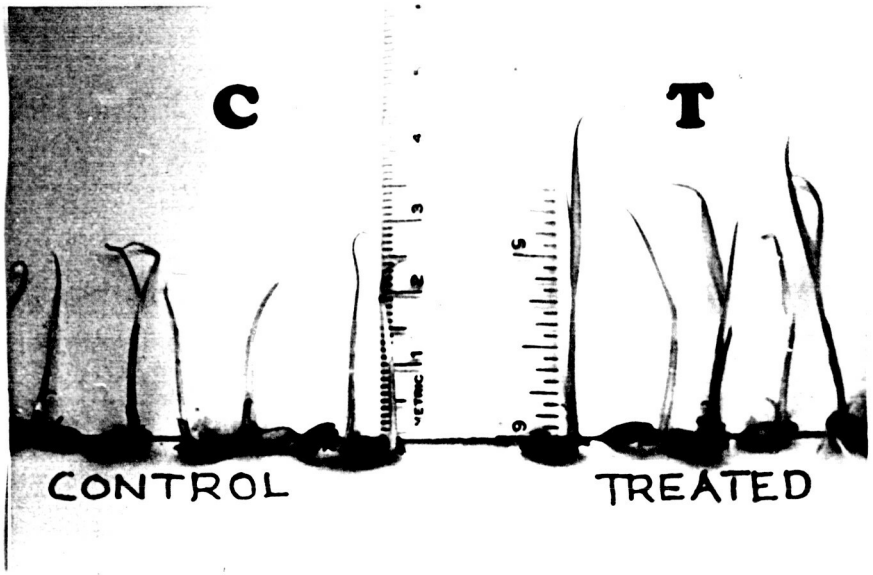


Fig. 2. Seedlings two weeks into germination. T = seedlings grown in the presence of SLS, C = seedlings grown in the absence of SLS.

2.2. Rice seed germination: Variety BG379-2:

The above experiment was duplicated to confirm the positive effect of SLS on seedling growth observed in the previous study.

A germination percentage of 100% was observed during this experiment in both the control and the treatment.

Seedling lengths were measured 14 days into germination and the data obtained are given in table 3.

Table 3.
Length of rice seedlings (in centimeters) of variety BG379-2 after 2 weeks:

CONTROL.		TREATMENT.	
Replicate 1	Replicate 2	Replicate 1	Replicate 2
4.8	3.9	3.0	4.2
3.9	3.5	3.0	4.2
3.4	2.6	3.9	3.3
3.6	3.8	2.8	4.2
3.0	3.5	2.1	3.6
2.8	3.7	2.1	3.3
3.0	3.2	3.9	2.9
3.2	4.0	2.9	3.8
3.4	2.5	2.8	3.3
3.1	3.5	2.6	2.8
3.0	2.0	3.1	2.8
1.5	2.1	3.1	2.5
2.5	2.1	3.2	2.3
2.9	3.0	3.0	2.4
2.4	2.5	3.1	1.0
3.1	1.9	3.1	1.8
1.1	3.2	2.5	1.1
3.2	1.0	1.6	1.6
2.6	0.1	2.5	1.1
3.9	2.5	2.0	0.2

ANOVA for the data of table 3 is given in APPENDIX 2 and no significant effect of SLS, as observed earlier, was observed on comparison of the treatment and the control.

2.3 Rice seed germination: Variety BG276-5:

Rice seeds of the variety BG276-5 were also germinated in the presence of 100 mg of SLS following the same procedure as above. Germination percentages obtained for the control and treatment, given in table 4, indicate neither inhibition nor promotion of SLS on germination.

Table 4.
Germination percentages for seeds of rice variety BG276-5:

CONTROL		TREATMENT	
Replicate 1	Replicate 2	Replicate 1	Replicate 2
65	90	80	85

Again seedling lengths were measured at two weeks of age and the data are given in table 5.

Table 5.
Length of seedlings (in centimeters) of rice variety BG276-5 at 2 weeks:

CONTROL:		TREATMENT:	
Replicate 1	Replicate 2	Replicate 1	Replicate 2
4.1	2.4	4.1	5.4
3.7	4.5	4.4	4.5
4.7	4.5	4.6	4.6
4.4	4.2	5.0	4.1
4.1	4.9	4.7	3.9
4.2	4.8	2.9	4.3
4.3	4.7	3.9	3.9
3.0	4.3	4.4	3.3
2.2	4.1	3.9	3.9
3.3	3.5	3.7	3.2
2.9	4.2	2.6	4.5
1.9	4.5	3.6	3.3
0.8	3.4	3.1	3.6
no germination	4.2	1.2	1.4
no germination	2.5	1.4	1.1
no germination	1.4	4.4	2.1
no germination	0.5	no germination	0.2
no germination	0.6	no germination	no germination

ANOVA for the table 5 (APPENDIX 3) indicate no significant effect of SLS on seedling growth compared to control.

2.4. Discussion of results:

One of the primary questions about SLS, that needs to be answered is, whether lunar soil can be used as a support medium for plant growth. Ideal support medium should not be toxic or inhibit seed germination and plant growth and development.

In all of the above studies, the germination percentages of rice seeds, both in the presence and absence of SLS were similar. This indicates that the presence of SLS did not effect the seed germination.

ANOVA tests on seedling growth data indicated a significant positive effect of SLS, in the first experiment but subsequent studies didnot confirm this observation. Yet, though statistically insignificant, the mean seedling length of rice seeds, germinated in the presence of SLS, was higher than that of control (Figures 3, 4 & 5) in all of the above studies.

Fig: 3

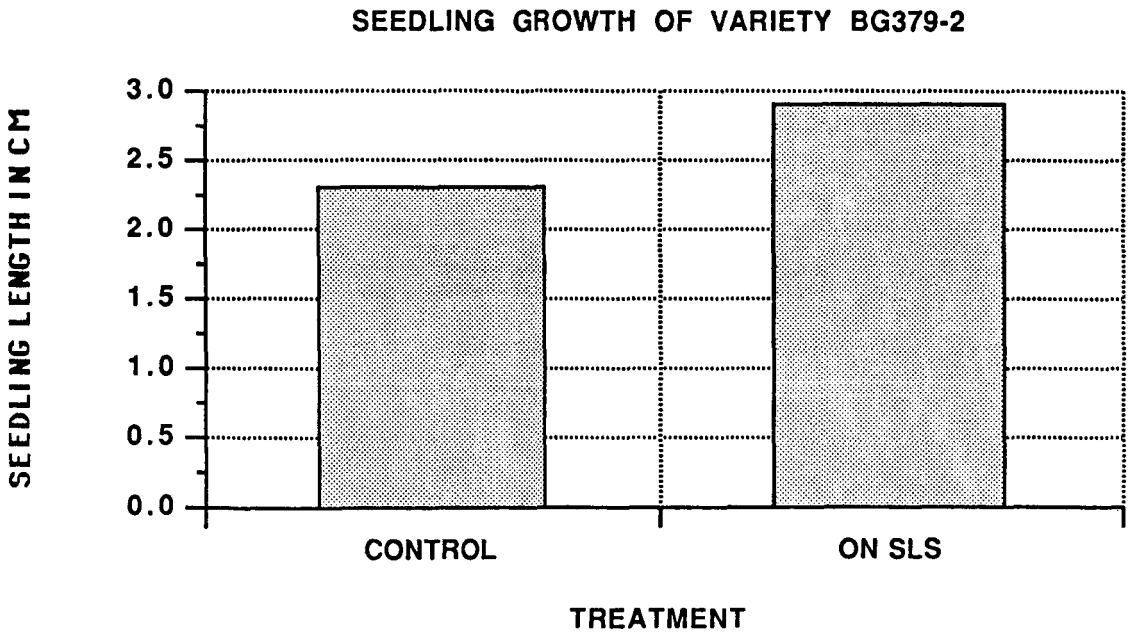


Fig: 4

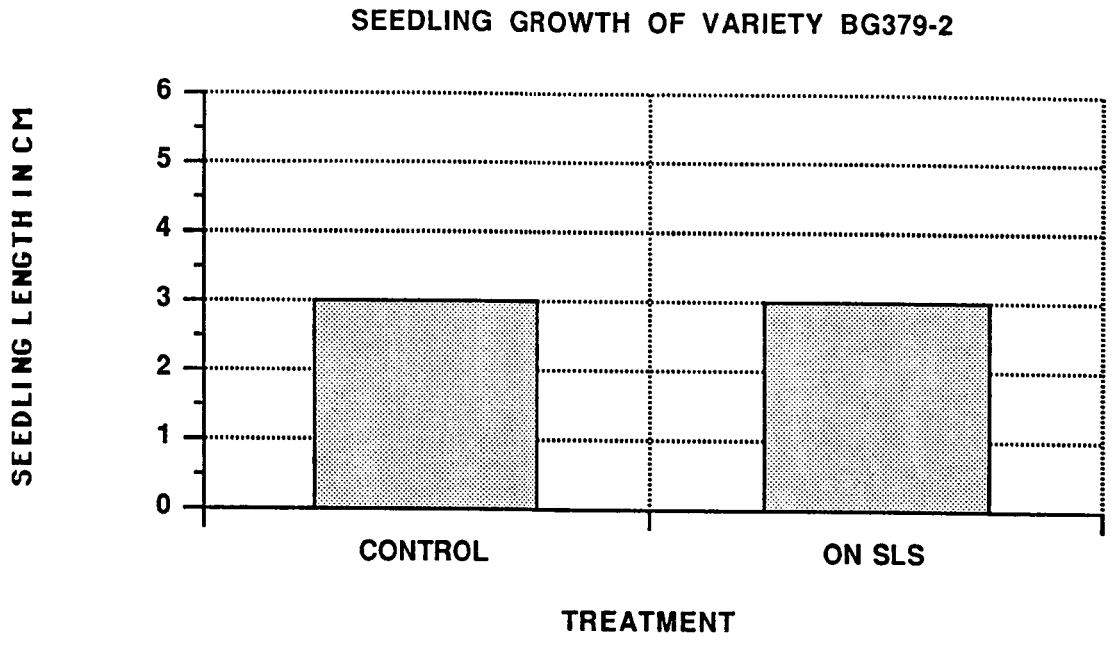
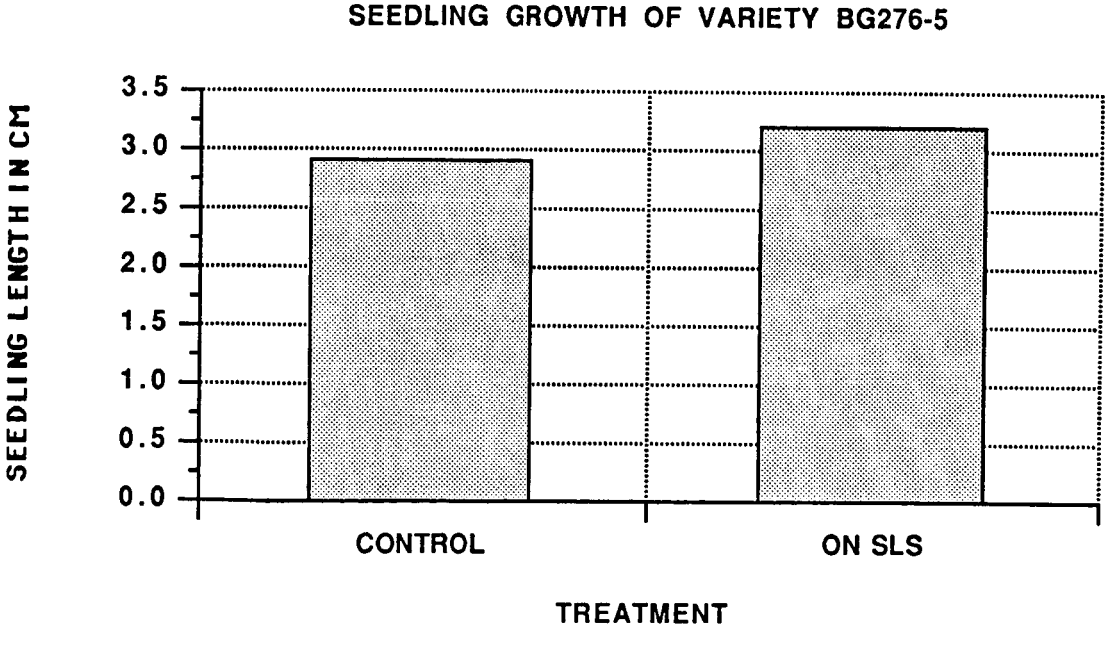

SEEDLING GROWTH OF VARIETY BG379-2
SEEDLING LENGTH IN CM
6
5
4
3
2
1
0
CONTROL
ON SLS
TREATMENT

Fig: 5


SEEDLING GROWTH OF VARIETY BG276-5
SEEDLING LENGTH IN CM
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CONTROL
ON SLS
TREATMENT

3.0 Tissue culture of winged bean (*Psophocarpus tetragonolobus* L. DC) and soybean (*Glycine max*) in the presence of Simulated Lunar Soil (SLS).

3.1. Effect of the placement of SLS in the medium on calli growth.

The experiment was designed to observe the effect of placement of SLS, in the medium, on winged bean and soybean callus tissue cultures.

The following three treatments and a control with no SLS, were set-up in a 'X' petri dish (Figure 6).

(1.) Calli were placed on 100mg of SLS, layered on top of agar.

(2.) The 100mg of SLS sprinkled on top of the calli, on the agar.

(3.) The 100mg of SLS mixed to the agar medium to be in the suspension.

A agar medium supplemented with Murashige Skoog salts (MS), 1 mg/ 1 naphthaleneacetic acid (NAA), 2 mg/1 2,4-dichlorophenoxyacetic acid (2,4-D) with 2.5% sugar and 3 pieces of calli, per replicate were used in all treatments (Figures 7 & 8). Winged bean and soybean callus tissues for the experiment were obtained from the cultures maintained in this laboratory. Each treatment was replicated four times.

Fresh weights of the calli were obtained at the beginning of the study, by weighing strictly under sterile conditions and the final fresh and dry weights were measured after one month of culture. The data obtained for winged bean and soybean are given in tables 6 & 7, respectively.

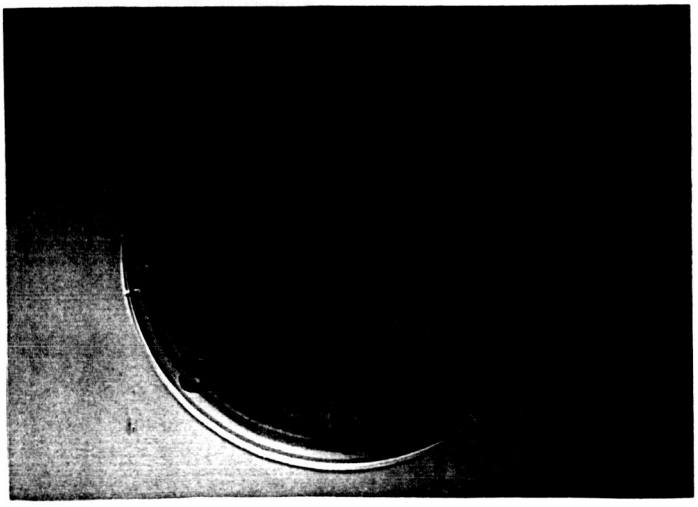


Fig. 6. 'X' plate used in the placement test. S = compartment with SLS in suspension in the medium.

Table 6.
Effect of the placement of SLS on winged bean calli growth:

TREATMENT	I.F.W.*	F.F.W.*	F.D.W.*	% D.W*	G.R.*
MS salts only	80.5	636.08	26.906	4.23	690.16
MS + SLS layer	81.3	590.08	24.84	4.21	625.8
MS + SLS sprinkled	82.7	613.8	28.787	4.69	642.2
SLS suspension in MS	85.75	738.6	25.629	3.47	761.34

* I.F.W. = Initial fresh weight, F.F.W. = Final fresh weight, F.D.W. = Final dry weight, % D.W. = percent of final dry weight/final fresh weight, G.R. = Growth rate measured as a percent of, increase of fresh weight/initial fresh weight. All weights are given in milligrams.

Table 7.
Effect of the placement of SLS on soybean calli growth:

TREATMENT	I.F.W.*	F.F.W.*	F.D.W.*	% D.W*	G.R.*
MS salts only	143.7	485	30.894	6.37	237.5
MS + SLS layer	125	449.6	24.098	5.36	259.68
MS + SLS sprinkled	106.7	372.7	25.269	6.78	249.29
SLS suspension in MS	126.4	454.75	21.737	4.78	259.77

* I.F.W. = Initial fresh weight, F.F.W. = Final fresh weight, F.D.W. = Final dry weight, % D.W. = percent of Final dry weight/final fresh weight, G.R. = Growth rate measured as a percent of, increase of fresh weight/initial fresh weight. All weights are given in milligrams.

3.2. Discussion of results:

Statistical analysis of varience (ANOVA) for growth rate data, obtained for winged bean is given in APPENDIX 4 and APPENDIX 5 contains the ANOVA tables for soybean.

Statistical comparison of the treatments indicate no significant effect on the growth rates of calli, between the treatments and the control, in both winged bean and soybean.

Even though, a higher weight increase for the calli grown in the medium with SLS in suspension, was obtained for both, soybean and winged bean (Figures 9 & 10), dry weight data indicate that this weight increase is more due to accumilation of water than actual growth.

This study confirms, the lack of toxicity or inhibition of SLS on plants, observed in the germination experiment.

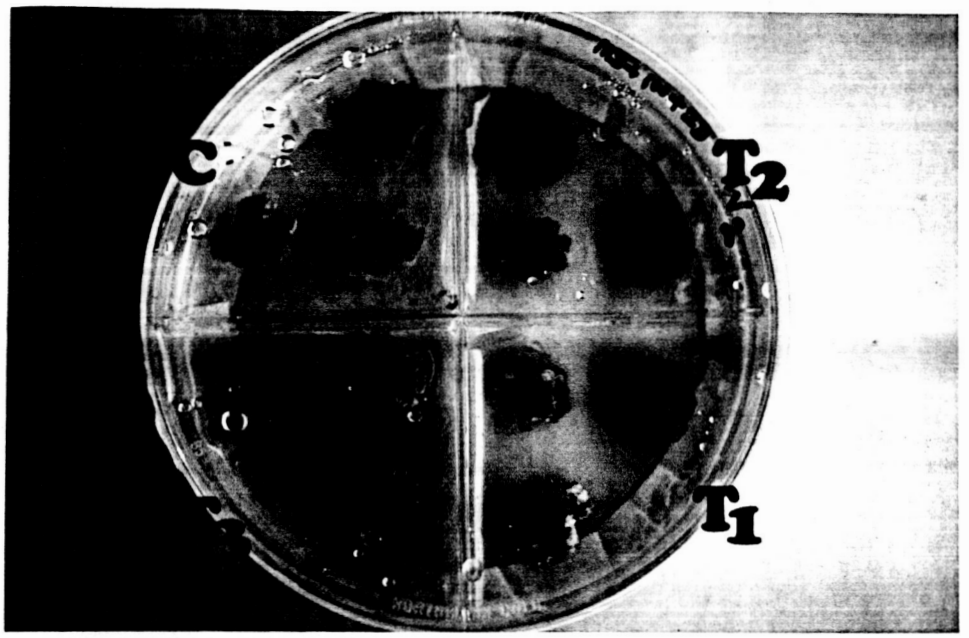


Fig. 7. Winged bean calli growing on 'X' plate in the 'Placement of SLS study'. T1 = calli on SLS layer, T2 = SLS sprinkled on calli, T3 = calli growing on medium with SLS in suspension, C = control

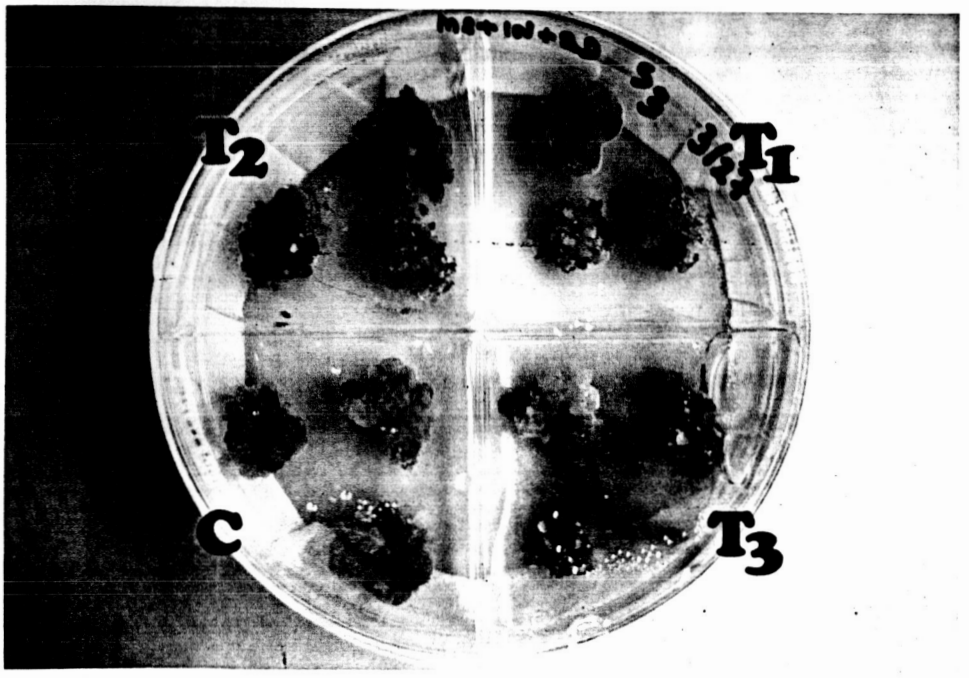


Fig. 8. Soybean calli growing on 'X' plate in the 'Placement of SLS study'. T1 = calli on SLS layer, T2 = SLS sprinkled on calli, T3 = calli growing on medium with SLS in suspension, C = control

Fig: 9.

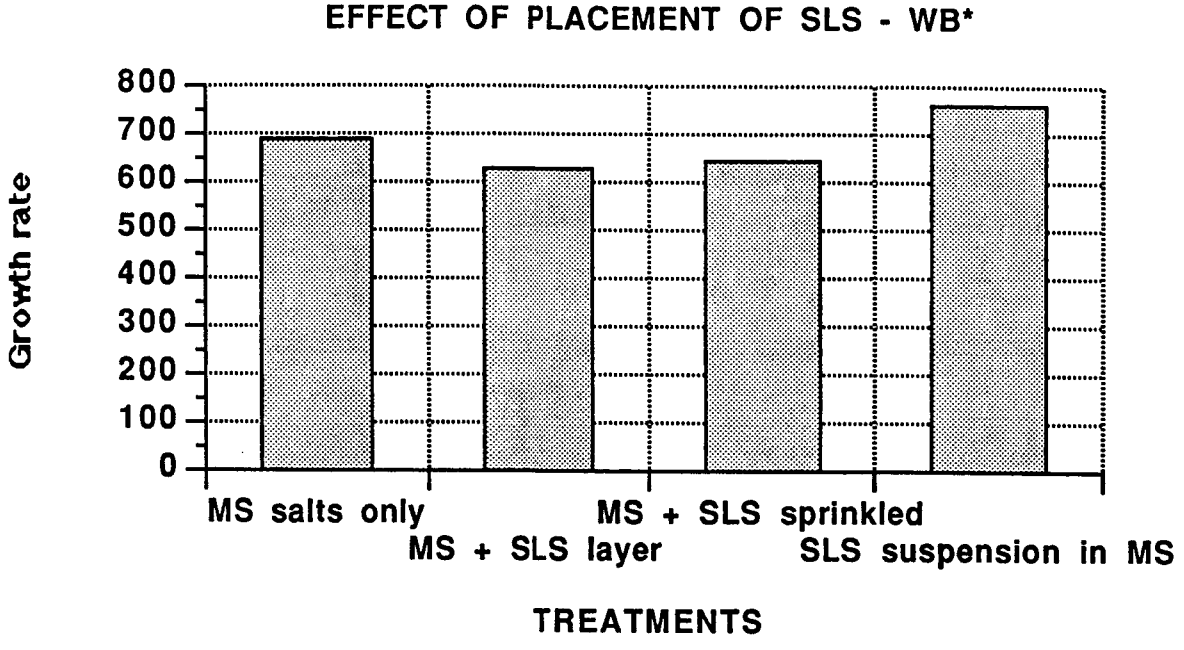
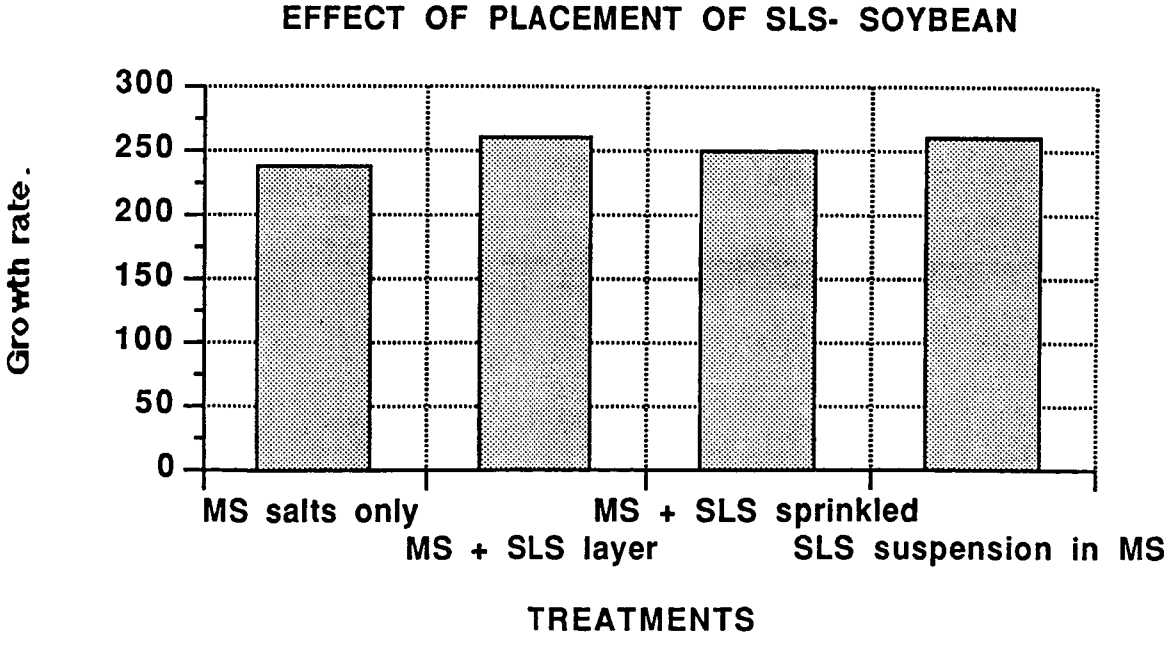


Fig: 10.



* WB = winged bean

3.3. Effect of the amount of SLS on growth of calli:

As no toxicity or inhibition of calli growth by SLS, was observed, during the previous experiments, this study was designed to determine the effect of SLS, at much higher concentrations and as a support medium for tissue culture.

Four levels of SLS in suspension were tested against media with no salts, MS salts and MS salts supplemented with 0.1% (weight/volume) SLS. The four levels were 0.1%, 0.2%, 0.4% and 0.8% on weight/volume basis, in suspension in agar in petri dishes. (This range was selected, as 0.1% SLS would supply the same amount of magnesium, an essential element of plants, as does the MS salts). Each treatment was replicated 4 times. All media were supplemented with 1 mg/l NAA and 1 mg/l 2,4-D.

Winged bean and soybean calli were grown for 1 month (figures 11 & 12) and fresh and dry weights of callus were measured at 7 day intervals. Dry weights for calli were not measured at the beginning of the study to avoid contamination. Fresh and dry weights obtained for winged bean are tabulated in tables 8 & 9 respectively. Data for soybean are given in tables 10 & 11.

Table 8.
Fresh weights of winged bean calli in milligrams:

WKS	MS only	no salts	MS.1%SLS	0.1%SLS	0.2%SLS	0.4%SLS	0.8%SLS
0	154.3	148.1	139.1	146.6	162.1	162.8	144.5
1	158	216	256	220	161	248	201
2	248	147	286	143	160	216.5	192
3	355	216.75	348.75	210	237.5	226.25	161.5
4	535	358.25	508	249.75	222.75	239.25	209.75

Table 9.
Dry weights of winged bean calli in milligrams:

WKS	MS only	no salts	MS.1%SLS	0.1%SLS	0.2%SLS	0.4%SLS	0.8%SLS
1	8	6	16	12	10	11	9
2	18	11	29.5	17.5	14.5	19	17.5
3	27.37	15.5	35.75	17.25	19.5	18.75	15.75
4	30.75	17	43.25	25.5	19	22.5	18.75

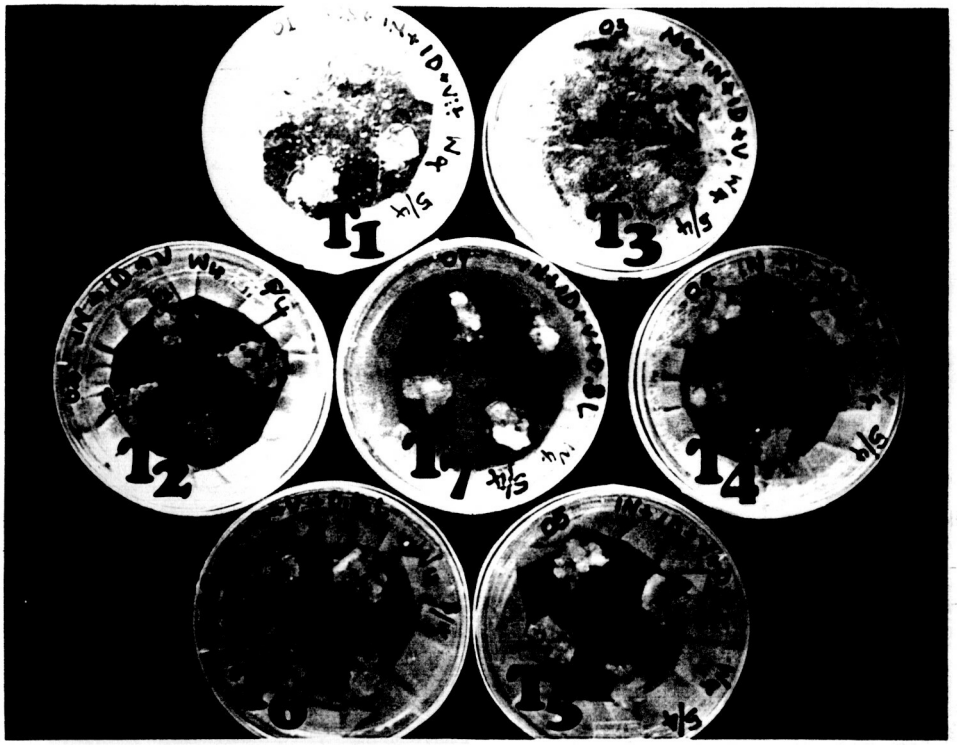


Fig. 11. Winged bean calli growing on MS only (T1), no salts (T2), MS + 0.1% SLS (T3), 0.1% SLS (T4), 0.2% SLS (T5), 0.4% SLS (T6) and 0.8% SLS (T7).

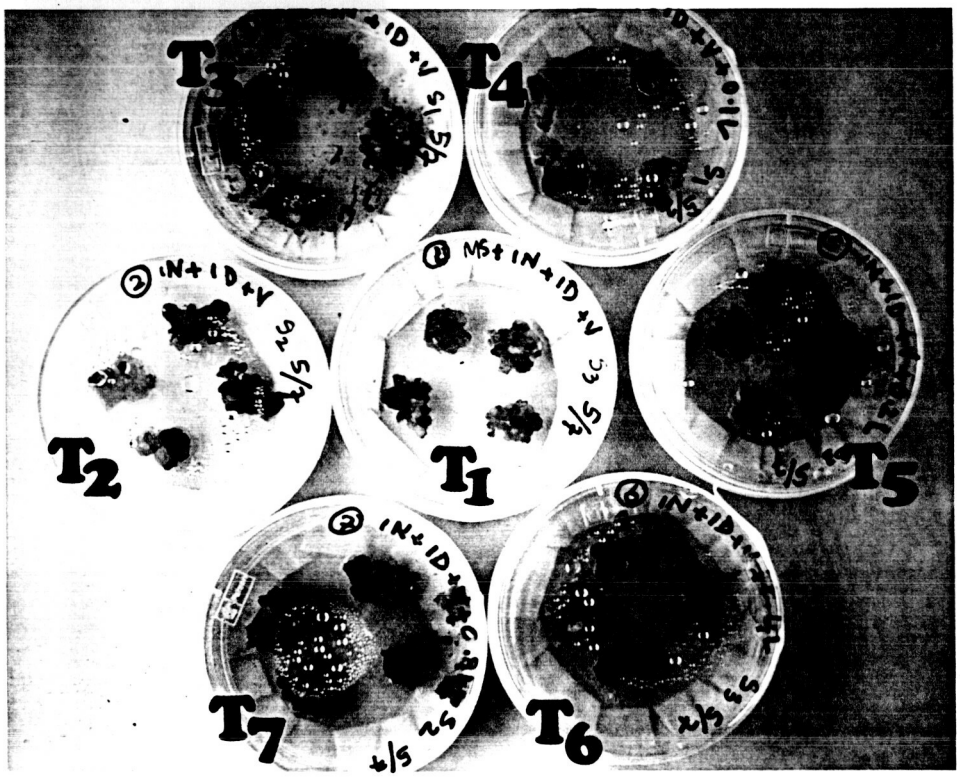


Fig. 12. Soybean calli growing on MS only (T1), no salts (T2), MS + 0.1% SLS (T3), 0.1% SLS (T4), 0.2% SLS (T5), 0.4% SLS (T6) and 0.8% SLS (T7).

Table 10.
Fresh weights of soybean calli in milligrams:

WKS	MS only	no salts	MS.1%SLS	0.1%SLS	0.2%SLS	0.4%SLS	0.8%SLS
0	219.4	224.9	222.75	227.4	224.5	233.25	223.8
1	275	315	289	307.5	274	247.5	252.3
2	634.3	312.3	585.3	362.3	281.3	309.3	263
3	468	364.7	595.3	348.3	424.7	475.3	365
4	808	352.5	468.3	375	777	387.75	480.25

Table 11.
Dry weights of soybean calli in milligrams:

WKS	MS only	no salts	MS.1%SLS	0.1%SLS	0.2%SLS	0.4%SLS	0.8%SLS
1	11.5	11.5	18	13	17	14	19.3
2	33.3	16.3	33.3	20	16	29.3	25.3
3	32.6	20.3	37.3	29	27	32.6	31.6
4	42.75	26.5	41.66	24.3	29.5	27.5	32.7

3.4. Discussion of results:

APPENDIX 6 and APPENDIX 7 contains the ANOVA tables compiled for data on winged bean and soybean, in that order.

Comparison of fresh weights, in ANOVA tables, between treatments, illustrates a significant drop in weight of calli grown on almost all levels of SLS, (except for 0.4% SLS level on winged bean and 0.2% SLS level of soybean) compared to calli grown on MS salts. This indicates that SLS, itself cannot support a culture system. Also, as no significant deviation is found between fresh weights of calli grown on MS salts and MS+0.1% SLS, statistically SLS behaves as a inert material.

Further, as there is no significant difference in fresh weights of calli grown on all levels of SLS and on media without any nutrient salts, the above observation is confirmed, for both winged bean and soybean.

Analysis of dry weights of both winged bean and soybean calli paints a different picture, of the effect of the amount of SLS on calli growth.

When the dry weights of calli grown on MS salts were compared with, dry weights obtained for calli grown on SLS only media, no significant difference could be found at the levels of 0.1% & 0.4% for

winged bean and 0.4% & 0.8% of soybean. This indicates that the calli grown on SLS too grew, at a similar rate as the calli nurtured by standard MS salts and that SLS was able to contribute the nutrients for calli growth.

This observation is confirmed by the significant difference of dry weights, at these level of SLS, when compared with media without any salts.

Further proof for the fact, that SLS contribute some nutrients for calli growth could be obtained from the comparison of dry weights for the calli grown on MS salts and MS + 0.1% SLS. A significant difference is seen with winged bean and statistically insignificant but higher dry weights are observed with soybean (figures 13 & 14).

The reason for lack of evidence for above observation, in fresh weight data could be that, SLS do not provide all the major elements, needed for plant growth and development. Analysis of the chemical composition of SLS support this, as SLS lack phophorus and nitrogen, two major elements needed by plants. Thus, the facts points that, SLS cannot support a tissue culture system on its own, but could be used as a support medium with supplements of certain specific major and micro elements.

Experiments to find the major and micro elements, that needs to be supplemented will be carried out in this lab.

Fig:13. EFFECT OF THE AMOUNT OF SLS ON CALLI GROWTH OF WINGED BEAN

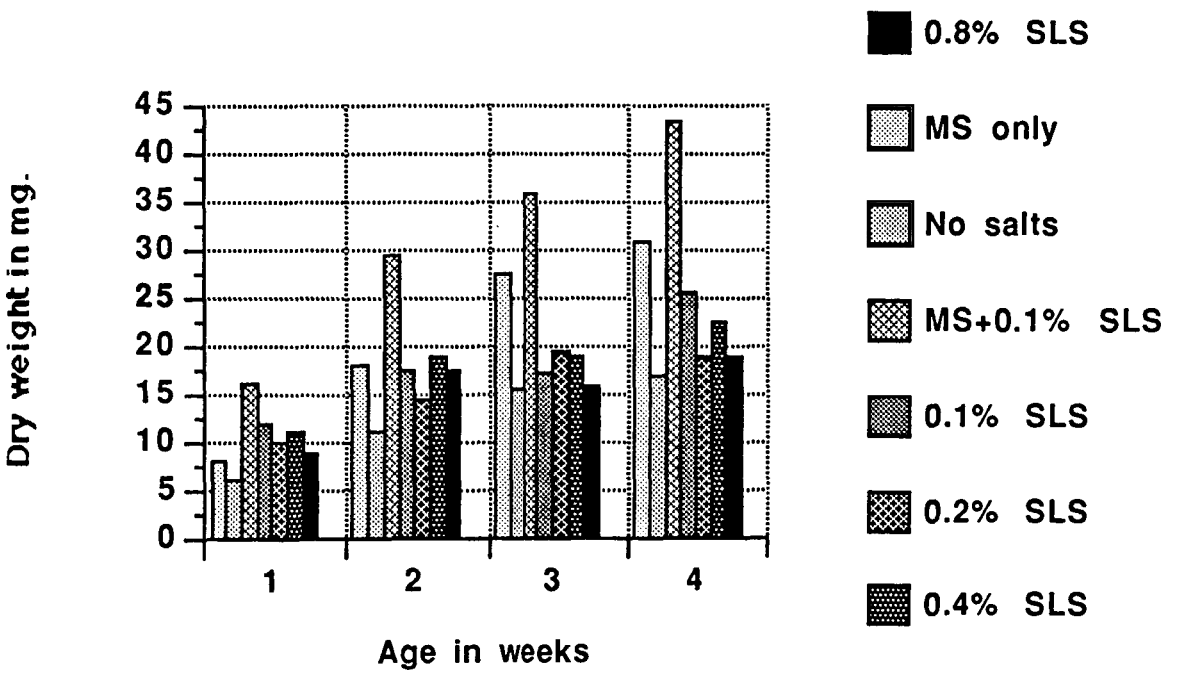
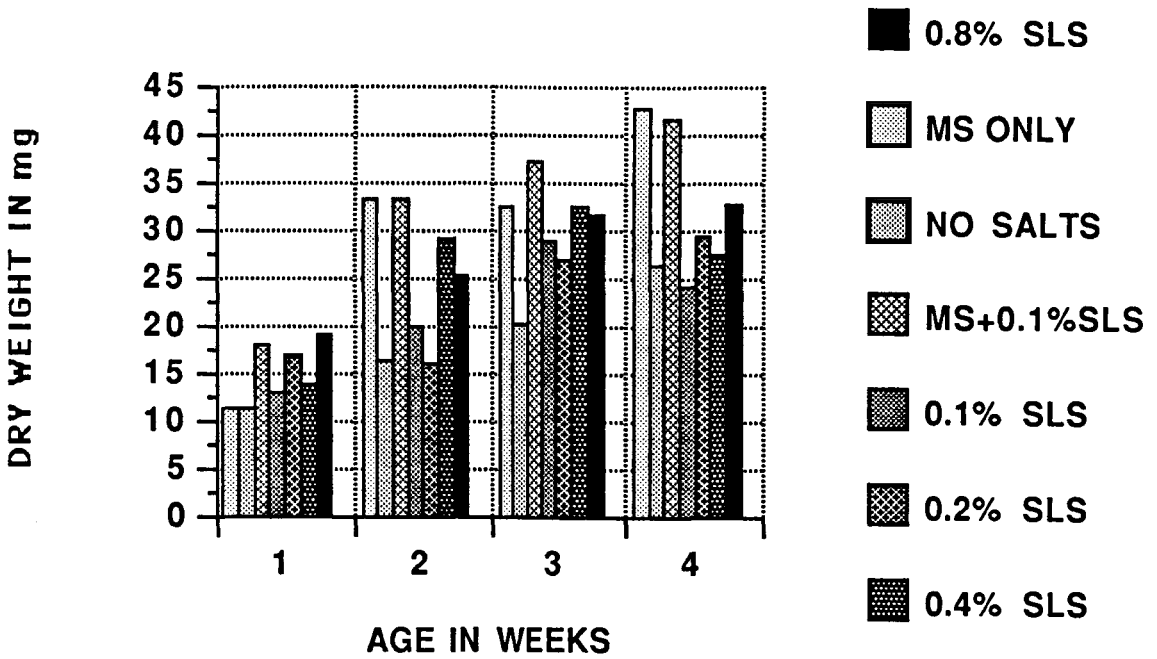


Fig.14. EFFECT OF THE AMOUNT OF SLS ON CALLI GROWTH OF SOYBEAN



4.0. CONCLUSION:

The rice seedling germination test initially indicated a significant effect of SLS on the seedling length, but subsequent experiments showed no sign of this effect. During all these studies, no sign of inhibition or toxicity or any other adverse effect of SLS on germination or on seedling elongation was observed.

The studies done to determine the best placement of SLS in the culture medium showed no difference between treatments, indicating that SLS could be placed either in contact or in suspension in the medium without any deleterious effect.

The experiments done to determine whether simulated lunar soil, itself could support calli growth indicated that, it could not nurture such a system, but was able to show a positive effect on growth rate of calli when supplemented with MS salts.

All the above studies were scaled down due to small amounts of SLS available and no experiments have been done to observe the effect of SLS in large quantities on seedling and calli growth. These experiments will be done once such quantities are made available.

In conclusion, Simulated Lunar Soil can be used as s support medium with supplements of certain specific major and micro elements.

APPENDIX 1
ANALYSIS OF VARIENCE FOR DATA OBTAINED FOR RICE SEED GERMINATION TEST. VARIETY BG 379-2.

EFFECT OF THE SLS ON RICE SEEDLING GROWTH - VARIETY BG379-2

One Factor ANOVA-Repeated Measures for X_1 ... X_2

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Between subjects	16	13.479	.842	1.201	.3549
Within subjects	17	11.92	.701		
treatments	1	3.305	3.305	6.137	.0248
residual	16	8.615	.538		
Total	33	25.399			

Reliability Estimates for- All treatments: .168 Single Treatment: .092

One Factor ANOVA-Repeated Measures for X_1 ... X_2

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
control	17	2.344	.451	.109
treatment	17	2.968	1.085	.263

One Factor ANOVA-Repeated Measures for X_1 ... X_2

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
control vs. treatment	-.624	.534*	6.137*	2.477

* Significant at 95%

APPENDIX 2
ANALYSIS OF VARIENCE FOR DATA OBTAINED FOR RICE SEED GERMINATION TEST. VARIETY BG 379-2.

EFFECT OF THE SLS ON RICE SEEDLING GROWTH - VARIETY BG379-2, 2ND TEST

One Factor ANOVA-Repeated Measures for X_1 ... X_2

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Between subjects	15	7.531	.502	4.478	.0025
Within subjects	16	1.794	.112		
treatments	1	.013	.013	.111	.7434
residual	15	1.781	.119		
Total	31	9.325			

Reliability Estimates for- All treatments: .777 Single Treatment: .635

One Factor ANOVA-Repeated Measures for X_1 ... X_2

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
control	16	3.044	.637	.159
treatment	16	3.003	.463	.116

One Factor ANOVA-Repeated Measures for X_1 ... X_2

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
control vs. treatment	.041	.26	.111	.334

APPENDIX 3
ANALYSIS OF VARIENCE FOR DATA OBTAINED FOR RICE SEED GERMINATION TEST. VARIETY BG 276-5.

EFFECT OF THE SLS ON RICE SEEDLING GROWTH - VARIETY BG276-5

One Factor ANOVA-Repeated Measures for X_1 ... X_2

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Between subjects	17	70.005	4.118	11.291	.0001
Within subjects	18	6.565	.365		
treatments	1	.49	.49	1.371	.2578
residual	17	6.075	.357		
Total	35	76.57			

Reliability Estimates for- All treatments: .911 Single Treatment: .837

One Factor ANOVA-Repeated Measures for X_1 ... X_2

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
control	18	2.967	1.496	.353
treatment	18	3.2	1.496	.353

One Factor ANOVA-Repeated Measures for X_1 ... X_2

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
control vs. treatment	-.233	.42	1.371	1.171

APPENDIX 4

ANALYSIS OF VARIENCE FOR DATA OBTAINED FOR STUDY ON THE EFFECT OF THE PLACEMENT OF SLS ON CALLI GROWTH OF WINGED BEAN.

EFFECT OF THE PLACEMENT OF SLS ON CALLI GROWTH OF WINGED BEAN

One Factor ANOVA-Repeated Measures for X_1 ... X_4

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Between subjects	3	127815.497	42605.166	1.439	.2802
Within subjects	12	355352.205	29612.684		
treatments	3	82801.337	27600.446	.911	.4732
residual	9	272550.868	30283.43		
Total	15	483167.702			

Reliability Estimates for- All treatments: .305 Single Treatment: .099

One Factor ANOVA-Repeated Measures for X_1 ... X_4

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
MS ONLY	4	729.5	209.788	104.894
MS + SLS LAYER	4	613.75	117.452	58.726
MS + SLS SPRINK...	4	641.75	101.369	50.684
SLS IN SUSPENSI...	4	795.364	255.683	127.842

One Factor ANOVA-Repeated Measures for X_1 ... X_4

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
MS ONLY vs. MS + SLS L...	115.75	278.396	.295	.941
MS ONLY vs. MS + SLS S...	87.75	278.396	.17	.713
MS ONLY vs. SLS IN SUSP...	-65.864	278.396	.096	.535
MS + SLS L... vs. MS + SL...	-28	278.396	.017	.228
MS + SLS L... vs. SLS IN ...	-181.614	278.396	.726	1.476

EFFECT OF THE PLACEMENT OF SLS ON CALLI GROWTH OF WINGED BEAN

One Factor ANOVA-Repeated Measures for X_1 ... X_4

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
MS + SLS S... vs. SLS IN ...	-153.614	278.396	.519	1.248

APPENDIX 5
ANALYSIS OF VARIENCE FOR DATA OBTAINED FOR STUDY ON THE EFFECT OF THE PLACEMENT OF SLS ON CALLI GROWTH OF SOYBEAN.

EFFECT OF THE PLACEMENT OF SLS ON CALLI GROWTH OF SOYBEAN

One Factor ANOVA-Repeated Measures for X_1 ... X_4

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Between subjects	3	24212.942	8070.981	2.167	.1449
Within subjects	12	44690.553	3724.213		
treatments	3	2028.508	676.169	.143	.9318
residual	9	42662.045	4740.227		
Total	15	68903.494			

Reliability Estimates for- All treatments: .539 Single Treatment: .226

One Factor ANOVA-Repeated Measures for X_1 ... X_4

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
MS SALTS ONLY	4	232.208	130.545	65.273
MS + SLS LAYER	4	260.49	45.265	22.633
MS + SLS SPRINK...	4	249.438	40.527	20.264
SLS IN SUSPENSI...	4	258.974	39.474	19.737

One Factor ANOVA-Repeated Measures for X_1 ... X_4

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
MS SALTS ... vs. MS + SL...	-28.282	110.144	.112	.581
MS SALTS ... vs. MS + SL...	-17.23	110.144	.042	.354
MS SALTS ... vs. SLS IN S...	-26.765	110.144	.101	.55
MS + SLS L... vs. MS + SL...	11.052	110.144	.017	.227
MS + SLS L... vs. SLS IN ...	1.517	110.144	3.235E-4	.031

EFFECT OF THE PLACEMENT OF SLS ON CALLI GROWTH OF SOYBEAN

One Factor ANOVA-Repeated Measures for X_1 ... X_4

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
MS + SLS S... vs. SLS IN ...	-9.535	110.144	.013	.196

APPENDIX 6
ANALYSIS OF VARIENCE FOR DATA OBTAINED FOR STUDY ON THE EFFECT OF THE AMOUNT OF SLS ON CALLI GROWTH OF WINGED BEAN.

EFFECT OF THE AMOUNT OF SLS ON CALLI GROWTH OF WINGED BEAN - FRESH WEIGHT

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Between subjects	4	129080.239	32270.06	5.558	.0018
Within subjects	30	174190.916	5806.364		
treatments	6	76192.53	12698.755	3.11	.0213
residual	24	97998.387	4083.266		
Total	34	303271.156			

Reliability Estimates for- All treatments: .82 Single Treatment: .394

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
MS ONLY	5	290.06	159.572	71.363
NO SALTS	5	217.22	86.023	38.471
MS+SLS	5	307.57	135.439	60.57
SLS0.1	5	193.87	47.137	21.08
SLS 0.2	5	188.67	38.208	17.087

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
SLS 0.4	5	218.56	33.419	14.945
SLS 0.8	5	181.75	27.646	12.364

EFFECT OF THE AMOUNT OF SLS ON CALLI GROWTH OF WINGED BEAN - FRESH WEIGHT

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
MS ONLY vs. NO SALTS	72.84	83.42	.541	1.802
MS ONLY vs. MS+SLS	-17.51	83.42	.031	.433
MS ONLY vs. SLS0.1	96.19	83.42*	.944	2.38
MS ONLY vs. SLS 0.2	101.39	83.42*	1.049	2.509
MS ONLY vs. SLS 0.4	71.5	83.42	.522	1.769

* Significant at 95%

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
MS ONLY vs. SLS 0.8	108.31	83.42*	1.197	2.68
NO SALTS vs. MS+SLS	-90.35	83.42*	.833	2.236
NO SALTS vs. SLS0.1	23.35	83.42	.056	.578
NO SALTS vs. SLS 0.2	28.55	83.42	.083	.706
NO SALTS vs. SLS 0.4	-1.34	83.42	1.832E-4	.033

* Significant at 95%

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
NO SALTS vs. SLS 0.8	35.47	83.42	.128	.878
MS+SLS vs. SLS0.1	113.7	83.42*	1.319	2.813
MS+SLS vs. SLS 0.2	118.9	83.42*	1.443	2.942
MS+SLS vs. SLS 0.4	89.01	83.42*	.808	2.202
MS+SLS vs. SLS 0.8	125.82	83.42*	1.615	3.113

* Significant at 95%

EFFECT OF THE AMOUNT OF SLS ON CALLI GROWTH OF WINGED BEAN - FRESH WEIGHT

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
SLS0.1 vs. SLS 0.2	5.2	83.42	.003	.129
SLS0.1 vs. SLS 0.4	-24.69	83.42	.062	.611
SLS0.1 vs. SLS 0.8	12.12	83.42	.015	.3
SLS 0.2 vs. SLS 0.4	-29.89	83.42	.091	.74
SLS 0.2 vs. SLS 0.8	6.92	83.42	.005	.171

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
SLS 0.4 vs. SLS 0.8	36.81	83.42	.138	.911

EFFECT OF THE AMOUNT OF SLS ON CALLI GROWTH OF WINGED BEAN - DRY WEIGHT

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Between subjects	3	849.355	283.118	6.156	.003
Within subjects	24	1103.686	45.987		
treatments	6	886.311	147.719	12.232	.0001
residual	18	217.375	12.076		
Total	27	1953.041			

Reliability Estimates for- All treatments: .838 Single Treatment: .424

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
MS ONLY	4	21.03	10.225	5.112
NO SALTS	4	12.375	4.956	2.478
MS+SLS	4	31.125	11.544	5.772
SLS0.1	4	18.062	5.569	2.785
SLS0.2	4	15.75	4.444	2.222

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
SLS0.4	4	17.812	4.854	2.427
SLS0.8	4	15.25	4.345	2.172

EFFECT OF THE AMOUNT OF SLS ON CALLI GROWTH OF WINGED BEAN - DRY WEIGHT

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
MS ONLY vs. NO SALTS	8.655	5.163*	2.068	3.522
MS ONLY vs. MS+SLS	-10.095	5.163*	2.813*	4.108
MS ONLY vs. SLS0.1	2.968	5.163	.243	1.208
MS ONLY vs. SLS0.2	5.28	5.163*	.77	2.149
MS ONLY vs. SLS0.4	3.218	5.163	.286	1.309

* Significant at 95%

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
MS ONLY vs. SLS0.8	5.78	5.163*	.922	2.352
NO SALTS vs. MS+SLS	-18.75	5.163*	9.704*	7.63
NO SALTS vs. SLS0.1	-5.688	5.163*	.893	2.315
NO SALTS vs. SLS0.2	-3.375	5.163	.314	1.373
NO SALTS vs. SLS0.4	-5.438	5.163*	.816	2.213

* Significant at 95%

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
NO SALTS vs. SLS0.8	-2.875	5.163	.228	1.17
MS+SLS vs. SLS0.1	13.062	5.163*	4.71*	5.316
MS+SLS vs. SLS0.2	15.375	5.163*	6.525*	6.257
MS+SLS vs. SLS0.4	13.312	5.163*	4.892*	5.418
MS+SLS vs. SLS0.8	15.875	5.163*	6.956*	6.46

* Significant at 95%

EFFECT OF THE AMOUNT OF SLS ON CALLI GROWTH OF WINGED BEAN - DRY WEIGHT

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
SLS0.1 vs. SLS0.2	2.312	5.163	.148	.941
SLS0.1 vs. SLS0.4	.25	5.163	.002	.102
SLS0.1 vs. SLS0.8	2.812	5.163	.218	1.145
SLS0.2 vs. SLS0.4	-2.062	5.163	.117	.839
SLS0.2 vs. SLS0.8	.5	5.163	.007	.203

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
SLS0.4 vs. SLS0.8	2.562	5.163	.181	1.043

APPENDIX 7

ANALYSIS OF VARIENCE FOR DATA OBTAINED FOR STUDY ON THE EFFECT OF THE AMOUNT OF SLS ON CALLI GROWTH OF SOYBEAN.

EFFECT OF THE AMOUNT OF SLS ON CALLI GROWTH OF SOYBEAN - FRESH WEIGHT

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Between subjects	4	396469.299	99117.325	7.27	.0003
Within subjects	30	409034.11	13634.47		
treatments	6	132455.7	22075.95	1.916	.1193
residual	24	276578.41	11524.1		
Total	34	805503.409			

Reliability Estimates for- All treatments: .862 Single Treatment: .472

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
MS ONLY	5	480.94	245.698	109.879
NO SALTS	5	313.88	54.762	24.49
MS+SLS	5	432.13	170.088	76.066
SLS0.1	5	324.1	59.712	26.704
SLS0.2	5	396.4	225.731	100.95

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
SLS0.4	5	330.62	101.292	45.299
SLS0.8	5	316.87	105.762	47.298

EFFECT OF THE AMOUNT OF SLS ON CALLI GROWTH OF SOYBEAN - FRESH WEIGHT

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
MS ONLY vs. NO SALTS	167.06	140.142*	1.009	2.461
MS ONLY vs. MS+SLS	48.81	140.142	.086	.719
MS ONLY vs. SLS0.1	156.84	140.142*	.889	2.31
MS ONLY vs. SLS0.2	84.54	140.142	.258	1.245
MS ONLY vs. SLS0.4	150.32	140.142*	.817	2.214

* Significant at 95%

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
MS ONLY vs. SLS0.8	164.07	140.142*	.973	2.417
NO SALTS vs. MS+SLS	-118.25	140.142	.506	1.742
NO SALTS vs. SLS0.1	-10.22	140.142	.004	.151
NO SALTS vs. SLS0.2	-82.52	140.142	.246	1.215
NO SALTS vs. SLS0.4	-16.74	140.142	.01	.247

* Significant at 95%

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
NO SALTS vs. SLS0.8	-2.99	140.142	3.232E-4	.044
MS+SLS vs. SLS0.1	108.03	140.142	.422	1.591
MS+SLS vs. SLS0.2	35.73	140.142	.046	.526
MS+SLS vs. SLS0.4	101.51	140.142	.373	1.495
MS+SLS vs. SLS0.8	115.26	140.142	.48	1.698

EFFECT OF THE AMOUNT OF SLS ON CALLI GROWTH OF SOYBEAN - FRESH WEIGHT

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
SLS0.1 vs. SLS0.2	-72.3	140.142	.189	1.065
SLS0.1 vs. SLS0.4	-6.52	140.142	.002	.096
SLS0.1 vs. SLS0.8	7.23	140.142	.002	.106
SLS0.2 vs. SLS0.4	65.78	140.142	.156	.969
SLS0.2 vs. SLS0.8	79.53	140.142	.229	1.171

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
SLS0.4 vs. SLS0.8	13.75	140.142	.007	.203

EFFECT OF THE AMOUNT OF SLS ON CALLI GROWTH OF SOYBEAN - DRY WEIGHT

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Between subjects	3	1243.801	414.6	11.088	.0001
Within subjects	24	897.435	37.393		
treatments	6	582.928	97.155	5.56	.0021
residual	18	314.507	17.473		
Total	27	2141.235			

Reliability Estimates for- All treatments: .91 Single Treatment: .59

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
MS ONLY	4	30.038	13.197	6.598
NO SALTS	4	18.65	6.351	3.175
MS+SLS	4	32.565	10.293	5.146
SLS0.1	4	21.575	6.796	3.398
SLS0.2	4	22.375	6.872	3.436

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
SLS0.4	4	25.85	8.177	4.089
SLS0.8	4	27.238	6.223	3.111

EFFECT OF THE AMOUNT OF SLS ON CALLI GROWTH OF SOYBEAN - DRY WEIGHT

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
MS ONLY vs. NO SALTS	11.387	6.21*	2.474	3.853
MS ONLY vs. MS+SLS	-2.527	6.21	.122	.855
MS ONLY vs. SLS0.1	8.462	6.21*	1.366	2.863
MS ONLY vs. SLS0.2	7.663	6.21*	1.12	2.592
MS ONLY vs. SLS0.4	4.187	6.21	.335	1.417

* Significant at 95%

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
MS ONLY vs. SLS0.8	2.8	6.21	.15	.947
NO SALTS vs. MS+SLS	-13.915	6.21*	3.694*	4.708
NO SALTS vs. SLS0.1	-2.925	6.21	.163	.99
NO SALTS vs. SLS0.2	-3.725	6.21	.265	1.26
NO SALTS vs. SLS0.4	-7.2	6.21*	.989	2.436

* Significant at 95%

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
NO SALTS vs. SLS0.8	-8.587	6.21*	1.407	2.905
MS+SLS vs. SLS0.1	10.99	6.21*	2.304	3.718
MS+SLS vs. SLS0.2	10.19	6.21*	1.981	3.448
MS+SLS vs. SLS0.4	6.715	6.21*	.86	2.272
MS+SLS vs. SLS0.8	5.327	6.21	.541	1.802

* Significant at 95%

EFFECT OF THE AMOUNT OF SLS ON CALLI GROWTH OF SOYBEAN - DRY WEIGHT

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
SLS0.1 vs. SLS0.2	-.8	6.21	.012	.271
SLS0.1 vs. SLS0.4	-4.275	6.21	.349	1.446
SLS0.1 vs. SLS0.8	-5.663	6.21	.612	1.916
SLS0.2 vs. SLS0.4	-3.475	6.21	.23	1.176
SLS0.2 vs. SLS0.8	-4.863	6.21	.451	1.645

One Factor ANOVA-Repeated Measures for X_1 ... X_7

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
SLS0.4 vs. SLS0.8	-1.387	6.21	.037	.469