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The effects of some selected herbicides and plant hormones on the growth of Prototheca wickerhamii

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LEAD CITRATE

a) Place 1,33g Pb $(NO_3)_2$ and 1,7g sodium citrate, with 30ml water, into a volumetric flask.
b) Shake for 1 min.
c) Leave for 30 min, shaking occassionally.
d) Add 8ml IN NaOH.
e) Make up to 50ml with distilled water.
f) If stored in the cold, the stain will keep for several months.

URANYL ACETATE

A saturated solution of uranyl acetate in distilled water is well shaken until the powder has dissolved. Make up a fresh solution for each staining.

APPENDIX iv

TABLE 1

NUMBER OF CELLS PER 0,1cc OF NUTRIENT BROTH UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

MEAN OF 5 SAMPLES

Time Hours	500 Lux 20°C	C Lux 32°C	2000 Lux 32°C
24	0,7	3,7	3,7
48	3,4	16,9	15,8
72	12,5	30,0	28,5
96	28,6	57,8	51,4
120	43,5	87,7	99,1
144	70,3	101,9	113,2
168	81,0	144,7	150,8

TABLE 2

NUMBER OF CELLS PER 0,1cc OF DIFFERENT NUTRIENT SOLUTIONS AT 2000 LUX AND 32°C

MEAN OF 6 SAMPLES

Time Hours	Nutrient Broth	Casselton and Stacey Solution with Glucose	with Sucrose
24	3,7	2,7	3,8
48	15,8	37,9	51,3
56	–	41,7	84,9
72	28,5	71,7	109,5
96	51,4	137,6	146,9
120	99,1	159,1	161,6
144	113,2	–	–
168	150,8	–	–

TABLE 3

ABSORBANCE AT 460mμ BY CELLS GROWN IN DIFFERENT NUTRIENT SOLUTIONS

MEAN OF 6 SAMPLES

Time Hours	Nutrient Broth	Casselton and Stacey Solution with Glucose	with Sucrose
24	,05	,10	,00
48	,16	,17	,16
72	,16	,20	,18
96	,22	,24	,21
120	,22	,26	,25
144	,26	,29	,26
168	,35	,39	,29

TABLE 4

MINIMUM SAMPLE SIZE REQUIREMENTS FOR HEMACYTOMETER

Time Hours	Nutrient Broth	Casselton and Stacey Solution with Glucose	with Sucrose
24	1	1	1
48	1	17	24
56	—	10	3
72	1	14	40
96	5	58	22
120	64	12	26
144	70	—	—
168	42	—	—

TABLE 5

MINIMUM SAMPLE SIZE REQUIREMENTS FOR SPECTROPHOTOMETER

Time Hours	Nutrient Broth	Casselton and Stacey Solution with Glucose	with Sucrose
24	5	11	13
48	31	20	44
72	8	58	77
96	6	7	1
120	2	2	37
144	1	4	118
168	8	5	39

APPENDIX v

TABLE 6

pH READINGS OF HERBICIDE SOLUTIONS

Herbicide	pH before Sterilisation	pH after Sterilisation	50% Herbicide 50% Nutrient Broth
MH	7,25	7,50	7,35
2,4,5-T	6,90	7,80	7,35

Herbicide	pH before Sterilisation	pH after Sterilisation	50% Herbicide 50% Nutrient Broth
NAA	6,70	7,50	7,35
IAA	7,00	7,30	7,35
IBA	7,00	6,90	7,35
IPA	7,30	7,00	7,30
Kinetin	7,00	7,50	7,30
GA_3	7,00	7,30	7,30
Simazine	7,00	7,20	7,30
Na Gold Salt	6,00	5,90	7,00
Coconut Milk	5,70	5,60	6,60

TABLE 7

EFFECT OF DIFFERENT HERBICIDES FROM 100 TO 1000µg/ml ON THE NUMBER OF CELLS PER 0,1cc

MEAN OF 5 SAMPLES

Herbicide	100	200	400	600	800	1000µg/ml
MH	56,0	52,0	52,8	54,9	53,0	55,0
2,4,5-T	57,4	50,9	54,7	52,7	54,5	57,3
NAA	48,8	51,0	55,0	55,5	59,0	53,8
IAA	42,9	30,7	25,3	24,6	21,8	21,5
IBA	33,6	28,1	21,7	23,3	23,6	21,1
IPA	30,3	29,0	22,8	24,7	23,8	21,6
GA_3	49,9	50,8	50,4	48,6	52,1	48,8
Simazine	48,2	49,7	47,7	51,4	49,7	52,9
Na Gold Salt	53,4	50,9	52,4	55,1	52,9	52,3

TABLE 8

CONTROL NUMBER OF CELLS PER 0,1cc FOR THE DIFFERENT HERBICIDES USED FROM 100 TO 1000µg/ml

MEAN OF 5 SAMPLES

Herbicide	48 hour Control	96 hour Control
MH	16,1	52,0
2,4,5-T	15,5	52,6
NAA	15,3	53,2
IAA	15,5	50,9
IBA	15,2	52,5
IPA	15,2	51,7
GA_3	15,2	50,3
Simazine	15,3	51,1
Na Gold Salt	15,3	52,5

TABLE 9

EFFECT OF DIFFERENT HERBICIDES FROM 10 TO 100µg/ml ON THE NUMBER OF CELLS PER 0,1cc

MEAN OF 5 SAMPLES

Herbicide	10	20	40	60	80	100µg/ml
Kinetin	51,2	49,5	51,0	49,4	49,1	51,1
IAA	50,7	48,8	51,2	47,2	43,6	43,8
IBA	55,5	53,4	43,2	40,9	41,1	34,1
IPA	54,5	50,0	44,6	44,1	41,7	42,0
Coconut	10%	20%	30%	40%	50%	v/v
Milk	55,0	54,9	55,7	53,5	87,3	

TABLE 10

CONTROL NUMBER OF CELLS PER 0,1cc FOR THE DIFFERENT HERBICIDES USED FROM 10 TO 100µg/ml

MEAN OF 5 SAMPLES

Herbicide	48 hour Control	96 hour Control
Kinetin	15,3	51,3
IAA	15,7	55,0
IBA	15,5	54,9
IPA	15,8	54,0
Coconut Milk	15,2	55,7

TABLE 11

EFFECT OF HIGHER CONCENTRATIONS OF SOME HERBICIDES ON THE NUMBER OF CELLS PER 0,1cc

MEAN OF 5 SAMPLES

Herbicide	2000µg/ml	4000µg/ml
MH	47,8	48,4
2,4,5-T	48,4	47,8
NAA	47,0	47,2
GA_3	48,2	48,4
Simazine	49,4	51,0
Control 48 hours	14,8	
Control 96 hours	47,9	

TABLE 12

TIME CURVES FOR HERBICIDES (NUMBER OF CELLS PER 0,1cc)

LIGHT/DARK PERIOD 7am TO 7pm

MEAN OF 3 SAMPLES

Time after Addition	IAA	IBA	IPA	Control
2 hours	24,2	27,0	24,8	25,3
4 hours	25,9	28,5	24,1	26,2
6 hours	27,8	29,8	27,3	27,9
8 hours	29,5	30,8	29,9	31,0
10 hours	31,3	30,8	31,4	32,2
12 hours	31,5	30,4	31,2	33,8
24 hours	32,3	30,3	32,7	43,6

TABLE 13

TIME CURVES FOR HERBICIDES (NUMBER OF CELLS PER 0,1cc)

LIGHT/DARK PERIOD 1am TO 1pm

MEAN OF 3 SAMPLES

Time after Addition	IAA	IBA	IPA	Control
2 hours	26,9	26,2	25,3	25,7
4 hours	28,7	27,9	26,9	27,7
6 hours	31,2	30,2	29,8	30,3
8 hours	31,9	33,8	31,0	31,4
10 hours	34,3	34,8	34,0	34,4
12 hours	35,6	34,4	34,0	36,3
24 hours	36,4	34,2	35,0	46,2

TABLE 14

PERCENTAGE INHIBITION OF PROTOTHECA WICKERHAMII WITH DIFFERENT HERBICIDES FROM 100 TO 1000µg/ml

Herbicide	100	200	400	600	800	1000µg/ml
MH	-12,9	0	-2,6	-9,4	-3,2	-9,7
2,4,5-T	-15,2	6,9	-5,7	-0,3	-6,0	-14,9
NAA	13,7	6,8	-5,6	-7,1	-18,0	-1,9
IAA	26,8	67,6	85,6	88,0	97,3	98,3
IBA	60,0	77,5	97,7	92,7	84,4	99,7
IPA	69,7	73,9	94,1	87,9	90,9	98,1
GA_3	1,4	-1,7	-0,3	5,8	-6,1	5,1
Simazine	9,6	4,7	11,3	-1,0	4,7	-6,0
Na Gold Salt	-2,9	5,1	0,3	-8,3	-1,3	1,0

TABLE 15

PERCENTAGE INHIBITION OF PROTOTHECA WICKERHAMII WITH DIFFERENT HERBICIDES FROM 10 TO 100µg/ml

Herbicide	10	20	40	60	80	100µg/ml
Kinetin	0,3	5,9	1,0	6,3	7,3	0,7
IAA	12,7	18,2	11,2	22,9	33,5	32,9
IBA	-1,8	4,4	34,5	41,3	35,4	61,4
IPA	-1,2	12,1	28,5	30,0	37,3	36,4
Coconut	10%	20%	30%	40%	50%	v/v
Milk	2,0	2,3	0	6,3	-91,1	

TABLE 16

PERCENTAGE INHIBITION WITH HIGHER CONCENTRATIONS OF SOME HERBICIDES

Herbicide	2000µg/ml	4000µg/ml
MH	4,3	2,1
2,4,5-T	2,1	4,3

Herbicide	2000µg/ml	4000µg/ml
NAA	7,1	6,4
GA_3	2,9	2,1
Simazine	-1,4	-7,1

APPENDIX vi

TABLE 17

TIME CURVE FOR IAA AT 400µg/ml (NUMBER OF CELLS PER 0,1cc)

MEAN OF 3 SAMPLES

Time after Addition	IAA	Control
2 hours	34,5	35,8
4 hours	36,0	36,8
6 hours	36,1	37,3
8 hours	37,1	37,7
10 hours	38,7	38,0
12 hours	38,4	38,5
24 hours	38,5	42,2

APPENDIX vii

TABLE 22

EFFECT OF HERBICIDES ON THE CELLULAR COMPONENT SIZES (µm) OF PROTOTHECA WICKERHAMII

Normal Cells			
Nucleus	3,7	±	1,1
Mitochondrion	1,3	±	0,6
Cell	10,9	±	4,9
Herbicide Treated Cells			
Nucleus	3,6	±	0,7
Mitochondrion	1,3	±	0,5
Cell	8,9	±	2,7

TABLE 23

DIFFERENCE BETWEEN MEANS TEST FOR NORMAL AND HERBICIDE TREATED CELLS

Nucleus diameter difference		t = ,241
At 26 d.f.	p <0,1	
Mitochondria length difference		t = ,091
At 127 d.f.	p <0,1	
Cell size difference		t = ,799
At 24 d.f.	p <0,1	

TABLE 24

PERCENTAGE DIVIDING AND DEGENERATING CELLS AS AFFECTED BY HERBICIDE TREATMENT (FIVE GRIDS)

Treatment	% Dividing	% Degenerating
Normal cells	13,19 ± 2,19	5,16 ± 0,63
Indoleacetic acid	11,06 ± 2,33	37,65 ± 9,92
Indolebutyric acid	9,23 ± 4,10	38,74 ± 9,18
Indolepropionic acid	13,46 ± 3,66	43,39 ± 2,65

APPENDIX viii

TABLE 26

INHIBITION MECHANISM STUDIES WITH REPLACEMENT OF HERBICIDE BY NUTRIENT BROTH (NUMBER OF CELLS PER 0,1cc)

MEAN OF 3 SAMPLES

Time (Hours)	x	10then 24	48	72	96
IAA	34,4	38,9	47,0	52,6	60,2	73,8
Control	35,9	39,7	45,3	51,4	60,2	78,4

Time (Hours)	x	15then 24	48	72	96
IAA	34,9	36,7	42,6	47,6	55,3	69,1
Control	36,3	43,0	52,3	53,4	62,3	78,2

Time (Hours)	x	20then 24		48	72	96
IAA	33,4	35,3	41,0	43,7	56,0	63,3
Control	34,1	40,1	50,1	57,7	71,9	83,0

TABLE 27

MODIFIED ENZYME KINETIC TYPE OF STUDY FOR SUBSTRATE CONCENTRATIONS 1,0; 0,75; 0,5 AND 0,25

MEAN OF 3 SAMPLES

WITH 1000µg/ml IAA

$\frac{1}{S}$	$\frac{1}{V}$	10 Hours	15 Hours	20 Hours
4,0		0,50	0,77	1,08
2,0		0,52	0,71	1,10
1,3		0,54	0,69	0,92
1,0		0,61	0,90	1,49

WITHOUT INHIBITOR

$\frac{1}{S}$	$\frac{1}{V}$	10 Hours	15 Hours	20 Hours
4,0		0,49	0,80	0,96
2,0		0,58	0,71	0,79
1,3		0,46	0,68	1,00
1,0		0,65	0,88	1,21

TABLE 28

MODIFIED ENZYME KINETIC TYPE OF STUDY FOR SUBSTRATE CONCENTRATIONS 1,0; 0,1; 0,01 AND 0,001

MEAN OF 3 SAMPLES

WITH 1000µg/ml IAA

$\frac{1}{S}$	$\frac{1}{V}$	10 Hours	15 Hours	20 Hours
1		0,20	0,30	0,39
10		0,20	0,29	0,37
100		0,29	0,41	0,54
1000		1,32	2,27	2,27

WITHOUT INHIBITOR

$\frac{1}{S}$	$\frac{1}{V}$	10 Hours	15 Hours	20 Hours
1		0,19	0,26	0,38
10		0,22	0,28	0,33
100		0,27	0,37	0,45
1000		0,99	1,11	1,39

TABLE 29

SIMPLE LINEAR REGRESSION ANALYSIS ON DATA FOR SUBSTRATE CONCENTRATIONS 1,0; 0,1; 0,01 AND 0,001

Treatment	Y Intercept $\frac{1}{V}$	$\bar{Y}$	Slope	Correlation Coefficient
10 Hours				
With 1000µg/ml IAA	0,19	0,50	0,001	1,000
Without inhibitor	0,20	0,42	0,001	1,000
15 Hours				
With 1000µg/ml IAA	0,26	0,82	0,002	0,999
Without inhibitor	0,27	0,51	0,001	1,000
20 Hours				
With 1000µg/ml IAA	0,36	0,89	0,002	1,000
Without inhibitor	0,35	0,64	0,001	0,999

$\bar{X}$ is 277,75. All r values significant ($p<0,001$).

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