JUNE

Week 2

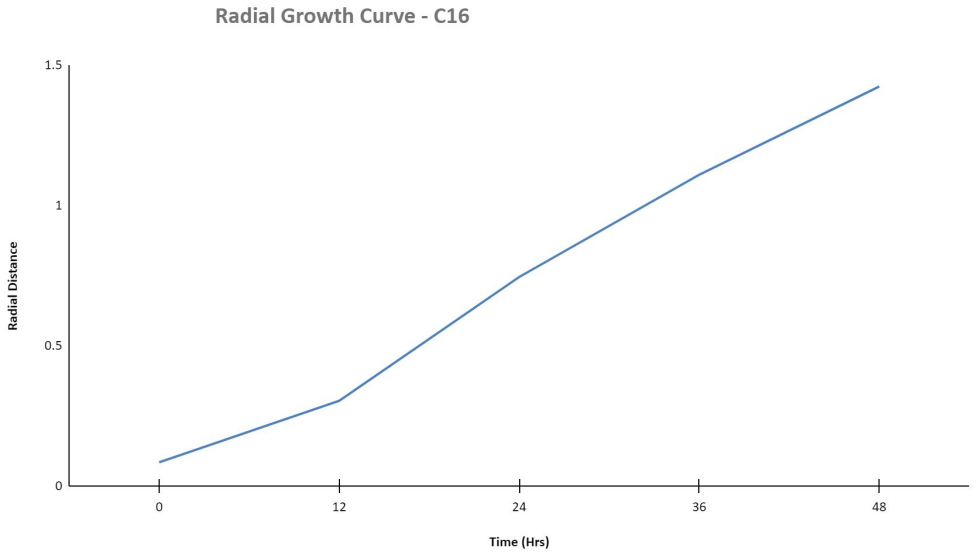
1. 9.06.2021-11.06.2021: We conducted **Radial growth experiments**.

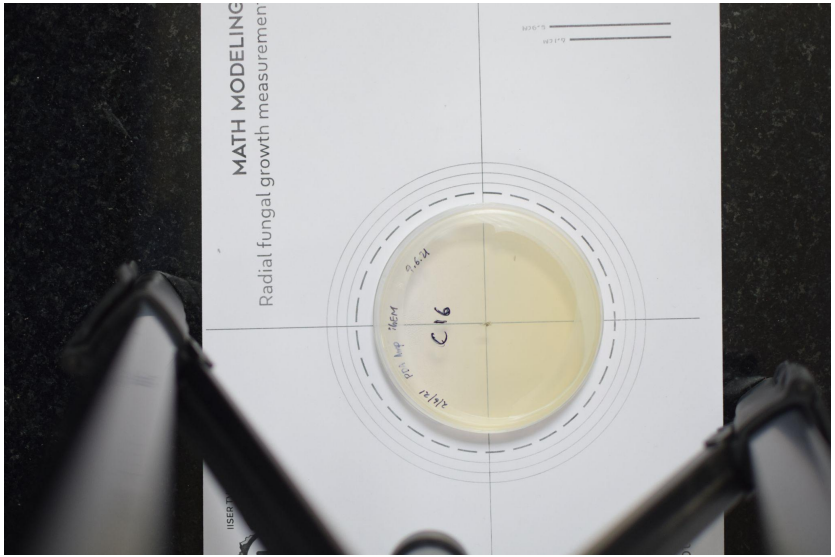
AIM: To analyse the growth of fungi by its changing radius.

a. Sample names unknown as fungal identification yet not done.
b. The fungal species were inoculated on PDA plates and sealed. They were kept at room temperature and images were taken at 6hr interval.
c. The curves were not smooth and not a distinguishable lag and exponential phase observed.

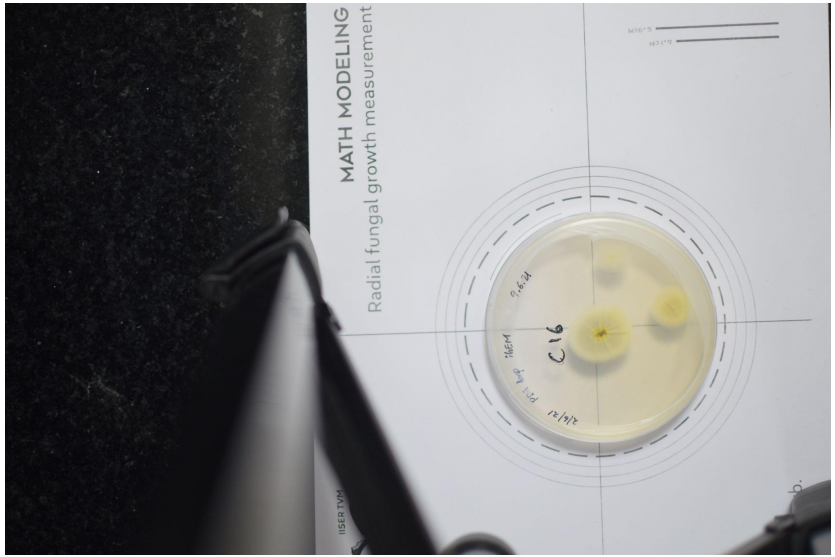
C16

Time(in hr)	Radius(cm)
0	0.085
12	0.304
24	0.745
36	1.109
48	1.423





0 hr

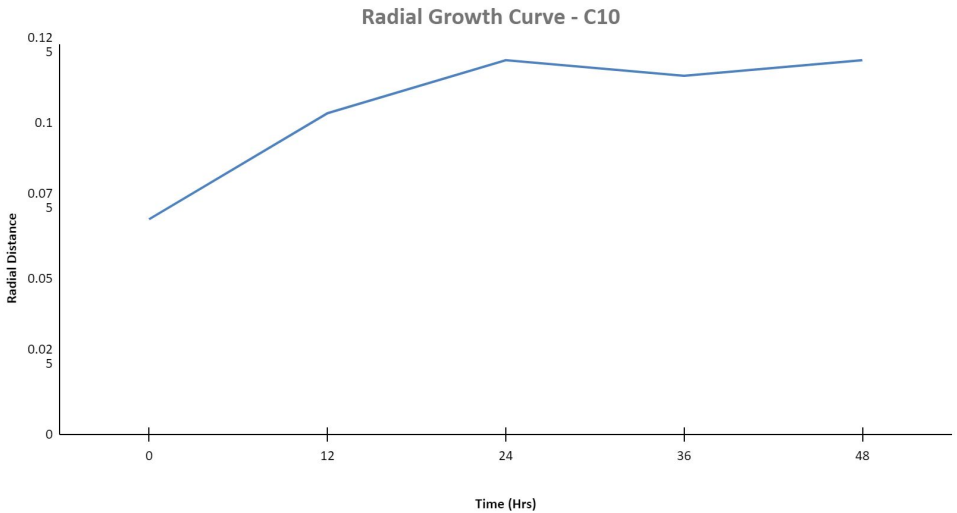


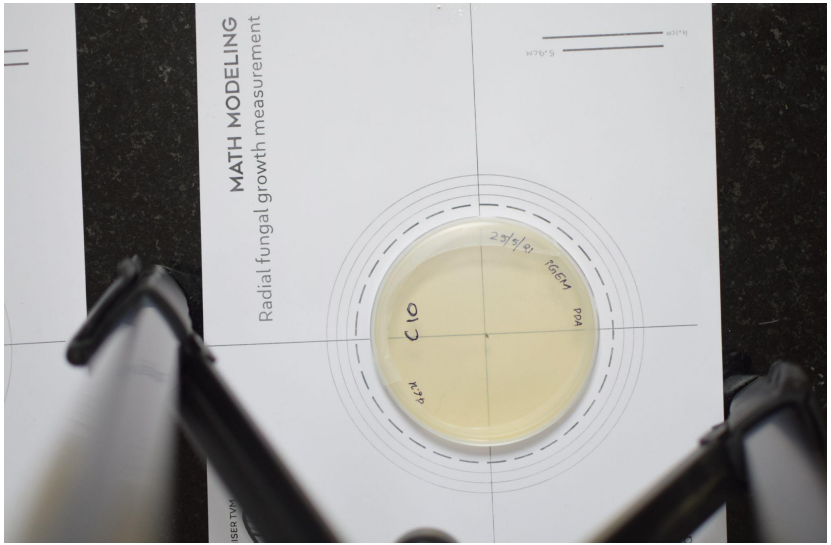
48 hr

Fig: Fungal culture - C16 on PDA petri plates

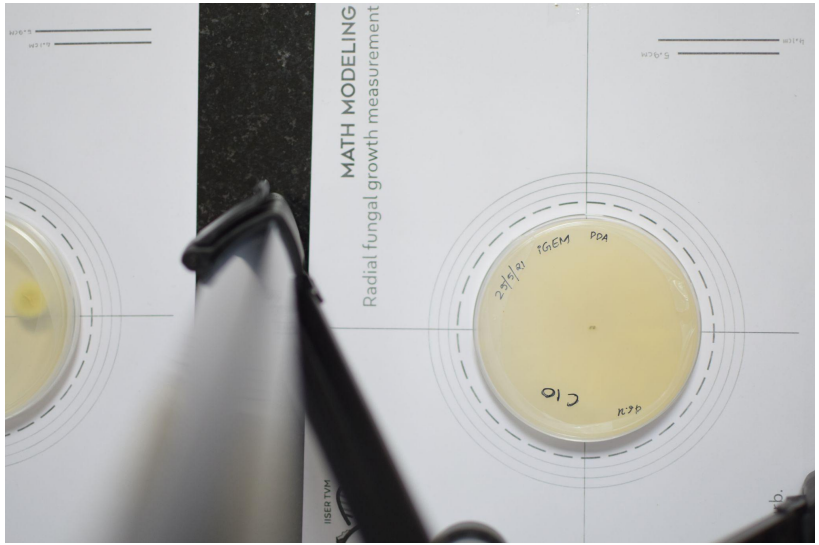
C10

Time(in hr)	Radius(cm)
0	0.069
12	0.103
24	0.12
36	0.115
48	0.12





0 hr

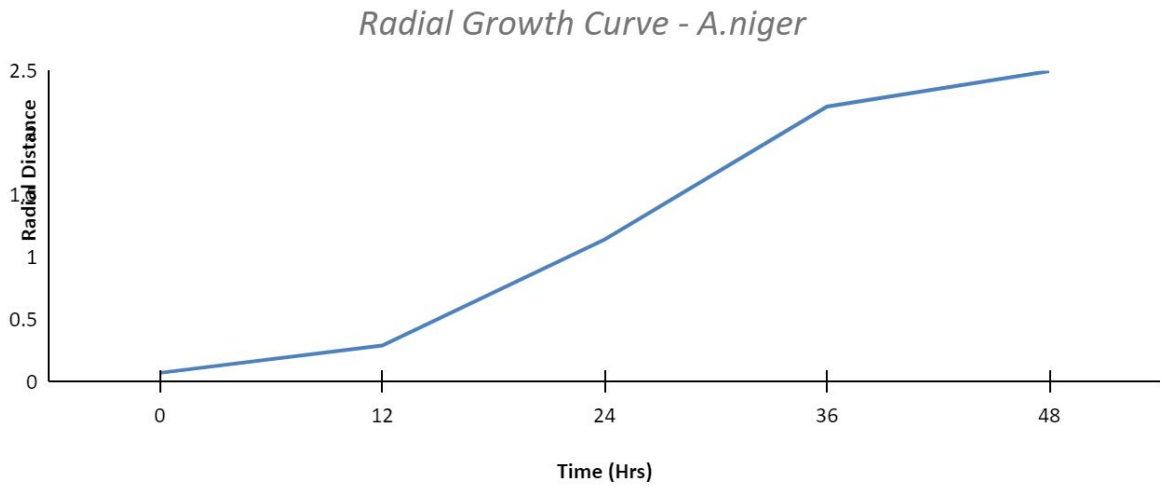


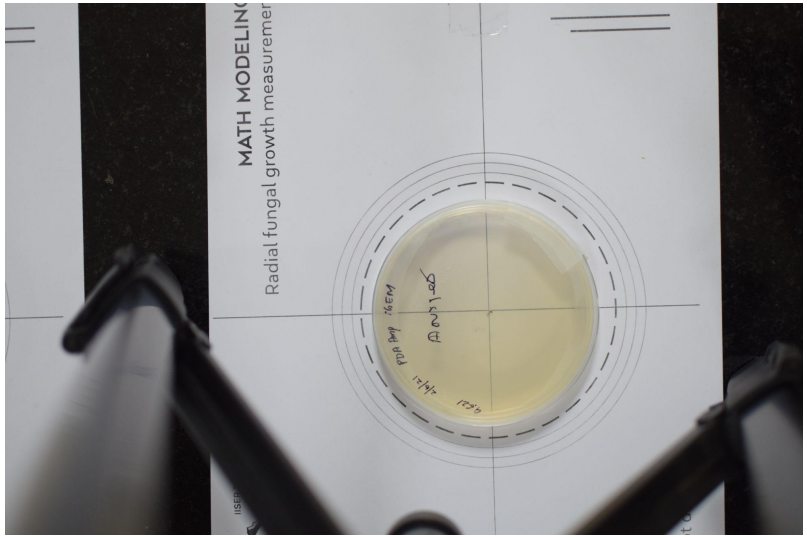
48 hr

Fig: Fungal culture - C10 on PDA petri plates

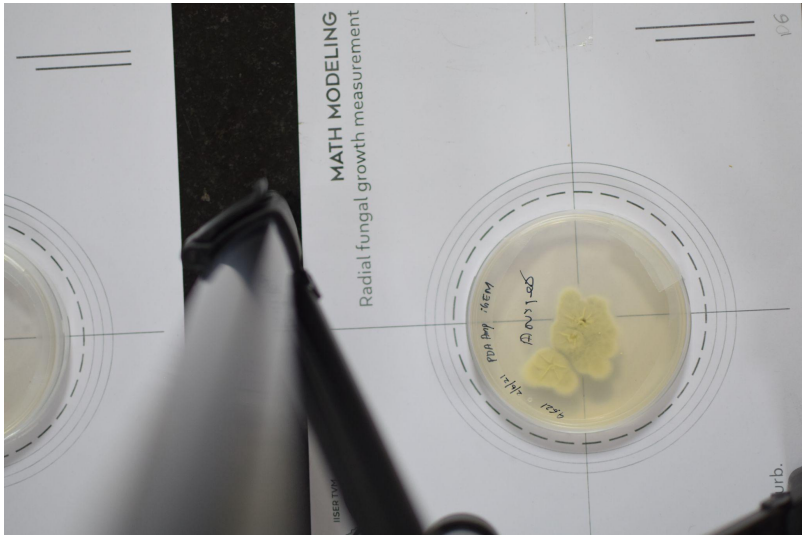
Aspergillus niger

Time (Hrs)	Radius(cm)
0	0.072
12	0.292
24	1.143
36	2.212
48	2.497





0 hr



48 hr

Fig: Fungal culture - *A.niger* on PDA petri plates

1. 9.06.2021-11.06.2021: **Absorbance data-Trial run** (600 nm)

   AIM:To analyse the growth of fungus by changing OD.

   a. Sample name unknown as fungal identification yet not done.
   b. The composition of sample in cuvette: 975uL of PDB + 25uL of spore suspension.
      Blank: 975uL of PDB + 25uL of PBS.
      Readings taken in spectrophotometer at 600nm
   c. Reading stopped after 11:15pm on 11.06.2021 as OD was too high to be measured.

Possible errors:
1. The spore concentration was too high.
2. The media concentration was not proper.
3. The lag phase is missed out due to long time intervals.

DAY	TIME	INTERVAL (hrs)	READING (A600)
09 - 06 - 2021	5 PM	-	0.119
10 - 06 - 2021	5 PM	24	1.631
10 - 06 - 2021	11 PM	6	2.087
11 - 06 - 2021	11:15 PM	12.25	NA

Week 3

1. 23.06.2021-30.06.2021: **Surface area-based growth experiment**

AIM**:** To analyse the growth of fungi by its changing radius and hence the changing surface area.

a. Sample names unknown as fungal identification not yet done.
b. The fungal species were inoculated on PDA plates and sealed. They were kept at room temperature and images were taken at 6hr interval.
c. Gompertz, Logistic and Richards models were fit and parameters found.
d. Gompertz was the best fit.

TABLE 1: Primary growth models.

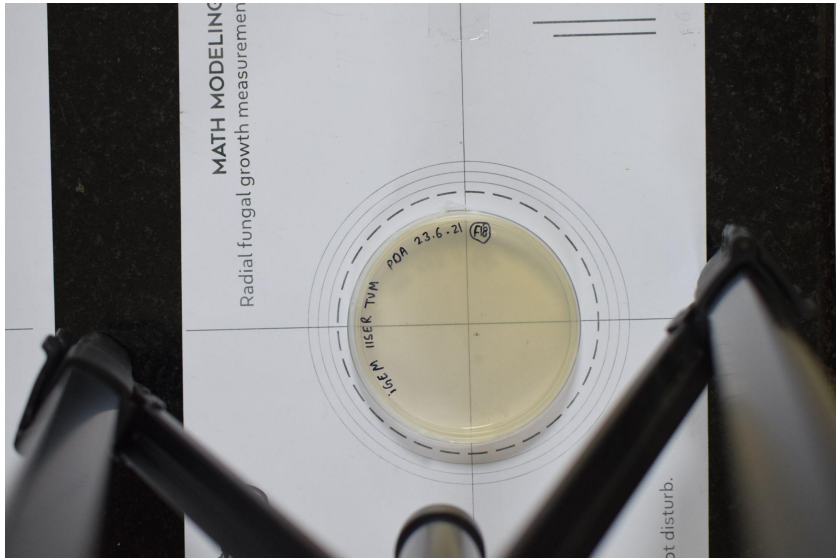
Model	Equation[a]
Three-phase linear	$y = y_0 \quad t \le \lambda$ $y = y_0 + \mu(t-\lambda) \quad \lambda < t < t_s$ $y = y_{max} \quad t \ge t_s$
Gompertz	$y = y_0 + C\left(e^{\left(-e^{(\mu e(\lambda - t)/C+1)}\right)}\right)$
Logistic	$y = y_0 + \frac{C}{1 + e^{(4\mu(\lambda - t)/C+2)}}$
Richards	$y = y_0 + \frac{C}{(1 + \beta e^{1+\beta} e^{((\mu/C)(1+\beta)(1+1/\beta)(\lambda - t))})^{1/\beta}}$
Baranyi	$y(t) = y_0 + \mu A(t) - \ln\left(1 + \frac{e^{\mu A(t)} - 1}{e^C}\right)$ $A(t) = t + \frac{1}{\mu}\ln\left(e^{-\mu t} + e^{-\mu\lambda} - e^{-\mu(t+\lambda)}\right)$

[a] y: log count or absorbance at time t; y_0: initial log count or absorbance; μ: maximum growth rate; λ: lag time; t_s: time to reach stationary growth phase; y_{max}: final log count or absorbance; C: increase in log count or absorbance from y_0 to y_{max}; β: model coefficient.

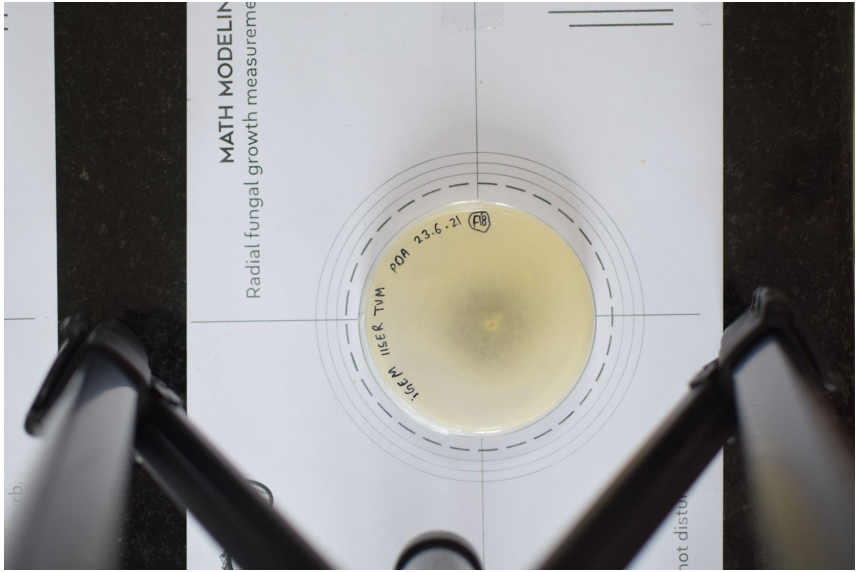
Note: Sample names have been renamed after the identification of the species.

Rhizopus oryzae

TIME (Hr)	AREA (cm^2)
0	0.047
6	0.067
12	3.508
18	13.949
24	38.797
42	69.432
48	85.968
54	88.317
60	89.527
66	90.003



0 hr



66 hr

Fig: Fungal culture - *R.oryzae* on PDA petri plates

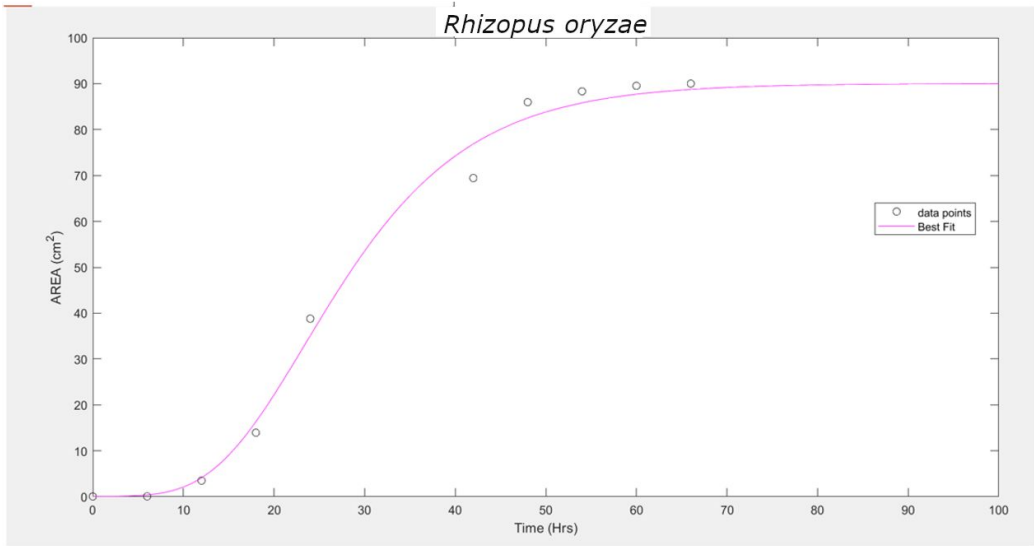
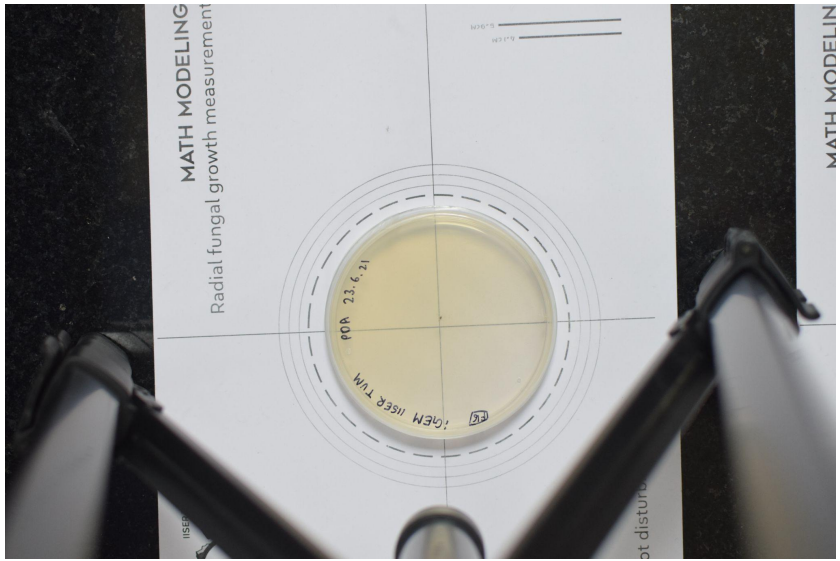


Fig: Gompertz fit of *R.oryzae*

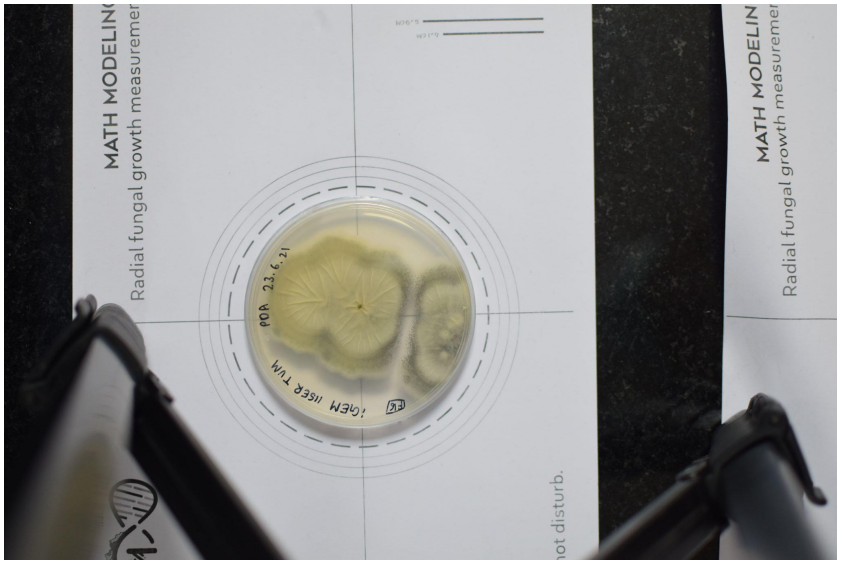
GOODNESS OF FIT	SSE	R-square	adjusted R-square	RMSE	u (max growth rate)	l (lag time)	B
Gompertz	98.28	0.9934	0.9925	3.505	3.319	23.42	
Richards	109.8	0.9926	0.9917	3.693	1.402	13.32	0.2167
Logistic	180.2	0.9879	0.9863	4.746	3.303	14.04	

Aspergillus niger

TIME (Hr)	AREA (cm^2)
0	0.018
6	0.025
12	0.14
18	0.456
24	1.156
42	7.027
48	11.16
54	12.601
60	14.741
66	17.441
72	19.029
78	20.202
90	23.569
96	24.896
102	25.363
120	26.593
126	26.659
138	26.876
144	27.012
150	27.014



0 hr



150 hr

Fig: Fungal culture - *A.niger* on PDA petri plates

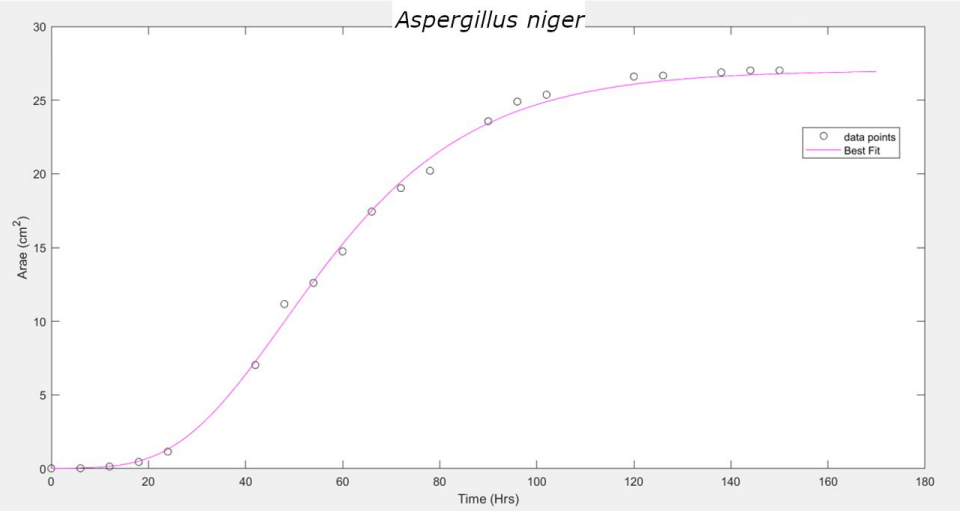
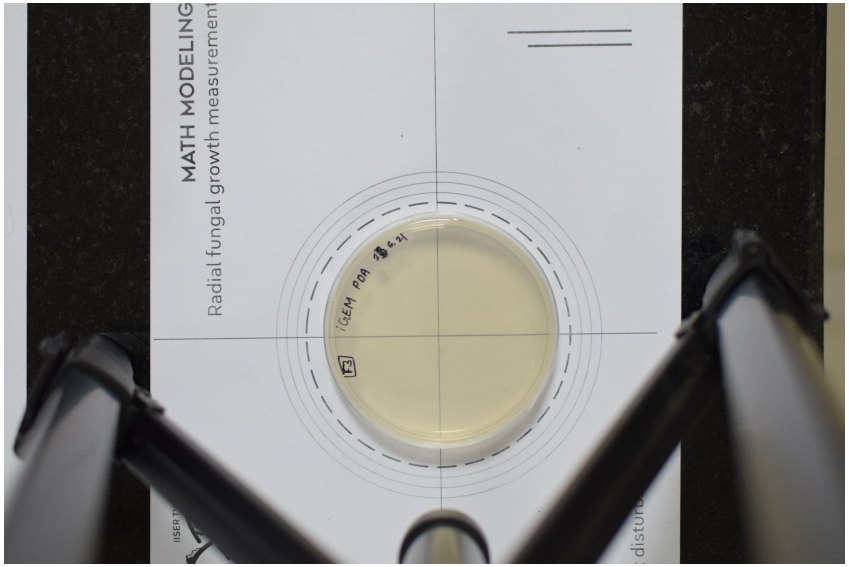


Fig: Gompertz fit of *A.niger*

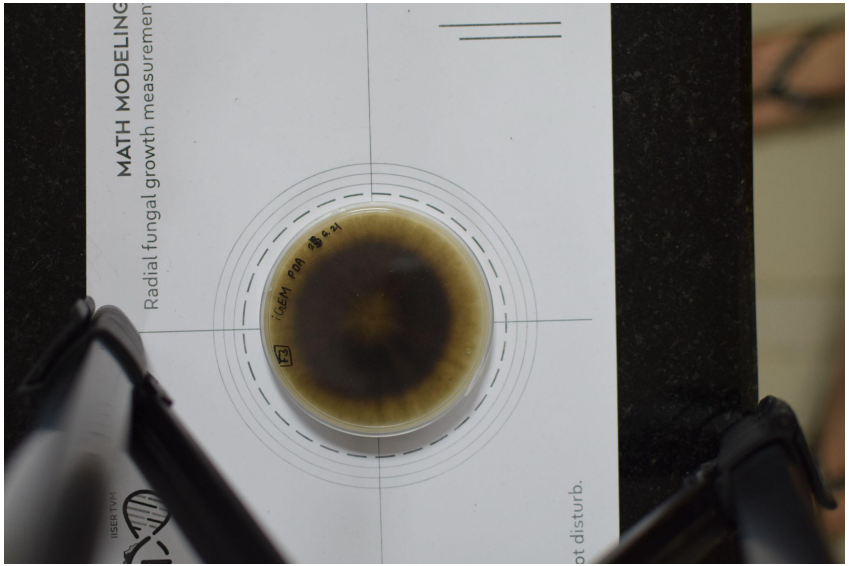
GOODNESS OF FIT	SSE	R-square	adjusted R-square	RMSE	u(max growth rate	l (lag time)	B
Gompertz	3.982	0.9982	0.9981	0.4704	0.4765	47.95	
Richards	3.951	0.9982	0.998	0.4821	0.05567	26.28	0.048
Logistic	12.64	0.9942	0.9939	0.8378	0.4471	27.46	

Nodulisporium indicum

Time (Hr)	AREA (cm^2)
0	0.014
6	0.028
12	0.05
18	0.063
24	0.073
42	1.344
48	5.421
54	8.222
60	12.321
66	16.85
72	25.327
78	30.064
90	33.829
96	38.449
102	43.298
120	43.485
126	46.47
138	47.542
144	48.132
150	49.137



0 hr



150 hr

Fig: Fungal culture - *N.indicum* on PDA petri plates

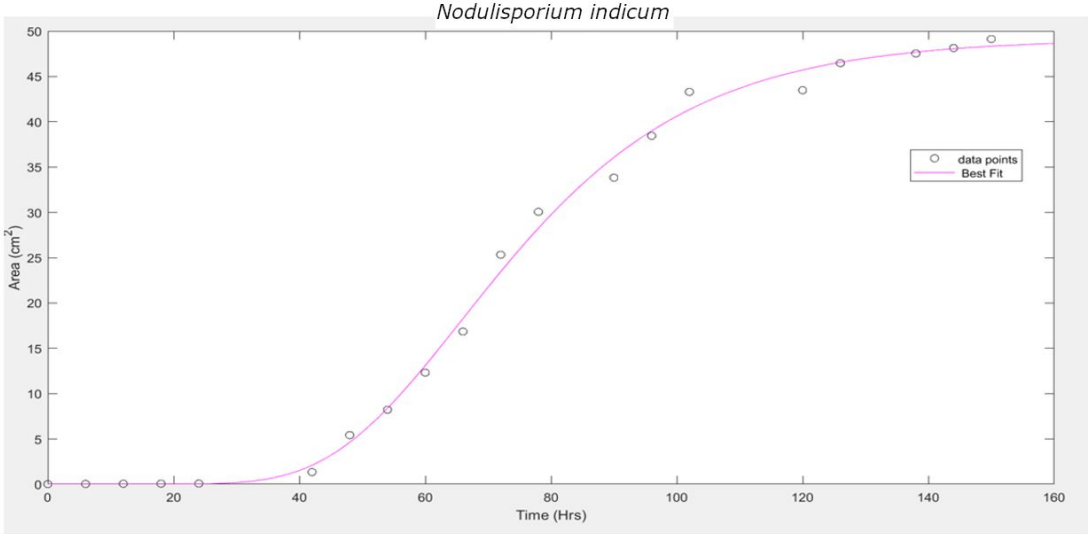
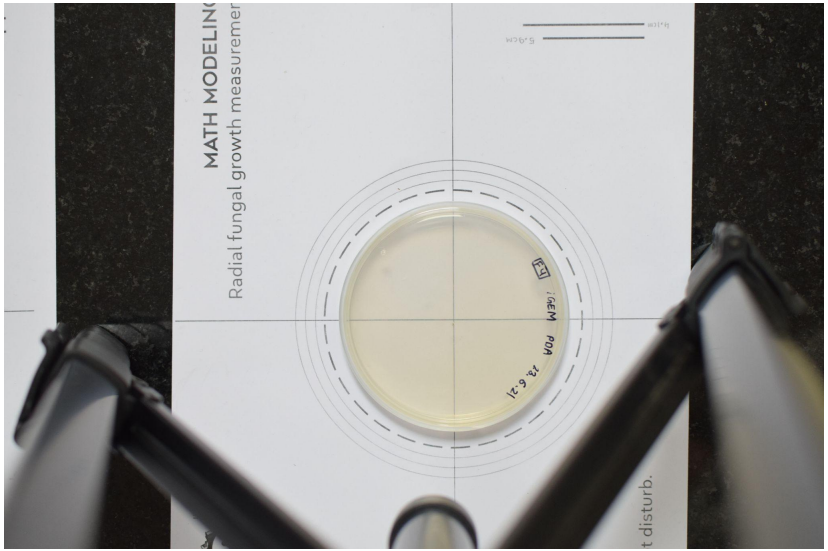


Fig: Gompertz fit of *N.Indicum*

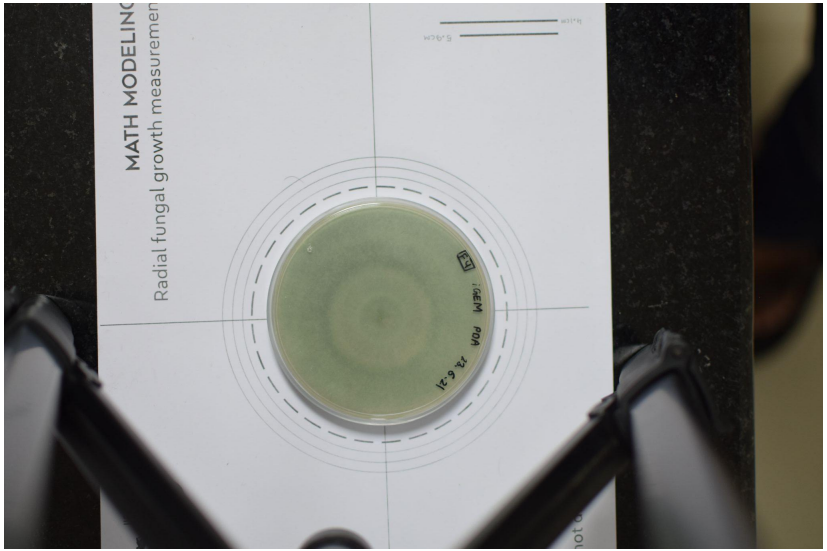
GOODNESS OF FIT	SSE	R-square	adjusted R- square	RMSE	u(max growth rate)	l (lag time)	B
Gompertz	25.43	0.9966	0.9964	1.188	0.8935	65.78	
Richards	28.72	0.9962	0.996	1.263	0.3809	45.27	0.2167
Logistic	60.19	0.992	0.9915	1.829	0.8587	46.42	

Trichoderma virens/harzianum

Time (Hr)	Area (cm^2)
0	0.019
6	0.02
12	1.071
18	3.851
24	8.918
42	36.802
48	46.971
54	57.534
60	65.833
66	77.465
72	79.612
78	82.201
90	83.934
96	84.002
102	84.102
120	84.149



0 hr



120 hr

Fig: Fungal culture - *Trichoderma spp.* on PDA petri plates

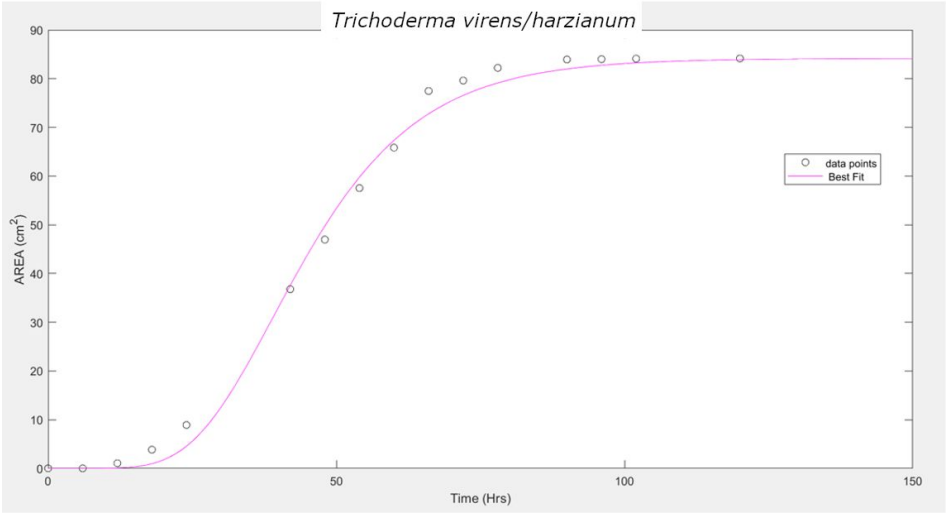
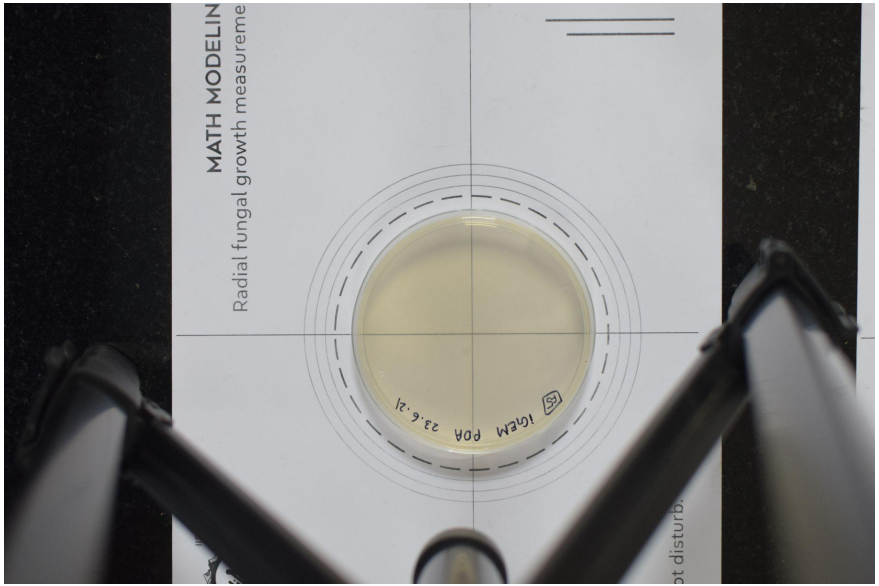


Fig: Gompertz fit of *Trichoderma spp.*

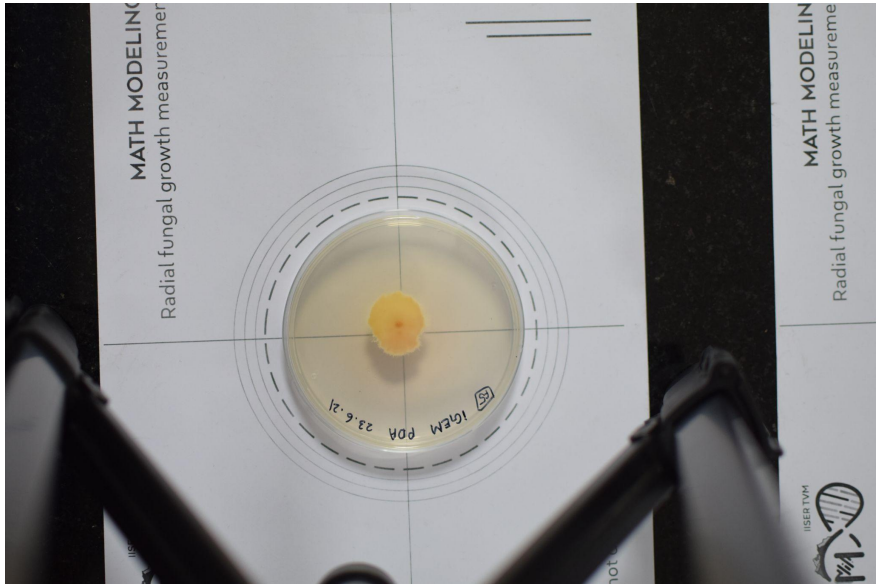
GOODNESS OF FIT	SSE	R-square	adjusted R-square	RMSE	u(max growth rate)	l(lag time)	B
Gompertz	90.91	0.9952	0.9949	2.548	2.238	39	
Richards	61.64	0.9968	0.9965	2.098	0.9522	24.97	0.2167
Logistic	30.26	0.9984	0.9983	1.47	2.152	26.03	

Fusarium solani

TIME (Hr)	AREA (cm^2)
0	0.005
6	0.007
12	0.062
18	0.149
24	0.546
42	1.819
48	2.769
54	3.611
60	4.028
66	4.113
72	4.376
78	4.489
90	5.071
96	5.152
102	5.191
120	5.207



0 hr



120 hr

Fig: Fungal culture - *F.solani* on PDA petri plates

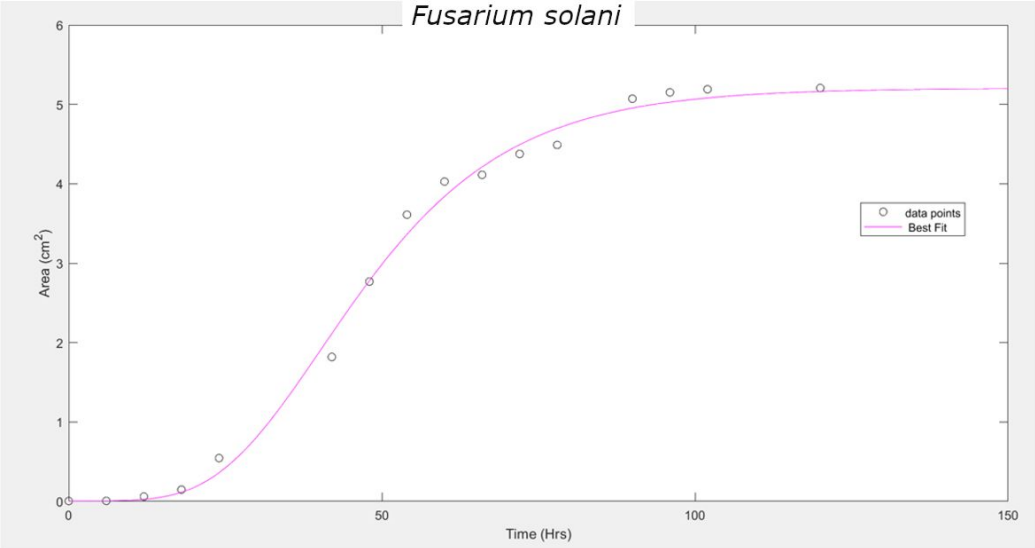


Fig: Gompertz fit of *F.solani*

GOODNESS OF FIT	SSE	R-square	adjusted R-square	RMSE	u (max growth rate)	l (lag time)	B(MODEL COEFF.)
Gompertz	0.3298	0.9951	0.9948	0.1535	0.1378	40.31	
Richards	0.3065	0.9955	0.9948	0.1535	0.05	23.86	0.2167
Logistic	0.5163	0.9923	0.9918	0.192	0.1143	25.06	

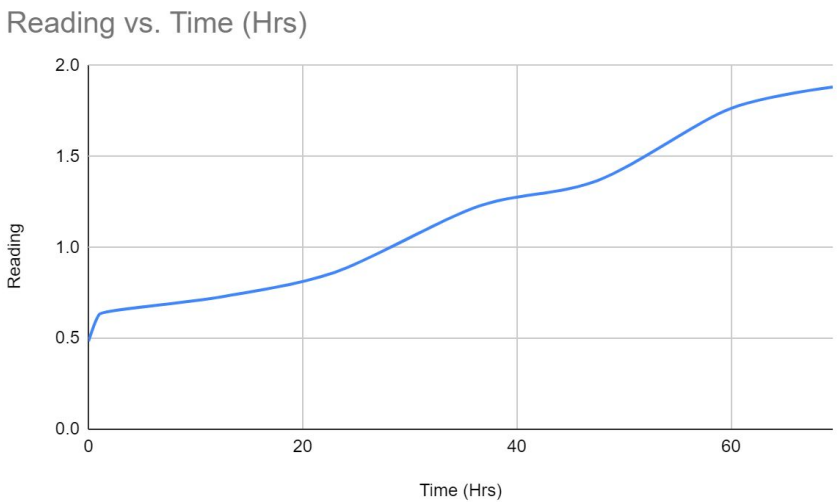
1. 26.06.2021-30.06.2021: Absorbance data-Trial run (600 nm)

AIM: To analyse the growth of fungi by its changing OD.

a. Sample name unknown as fungal identification not yet done.
b. The composition of sample in cuvette: 990uL of PDB + 10uL of spore suspension.
Blank: 990uL of PDB + 10uL of PBS.
Readings taken in spectrophotometer at 600nm

c. The curve did not have a distinguishable lag and exponential phase.

Date	Time	Interval(hr)	Reading
26/06/2021	11pm	0	1.905
27/06/2021	12am	1	0.484
27/06/2021	1am	1	0.632
27/06/2021	12:30pm	11.5	0.729
27/06/2021	11pm	10.5	0.863
28/06/2021	12:30pm	13.5	1.228
28/06/2021	11:30pm	11	1.367
30/06/2021	12pm	12.5	1.765
30/06/2021	9.30pm	9.5	1.882



JULY

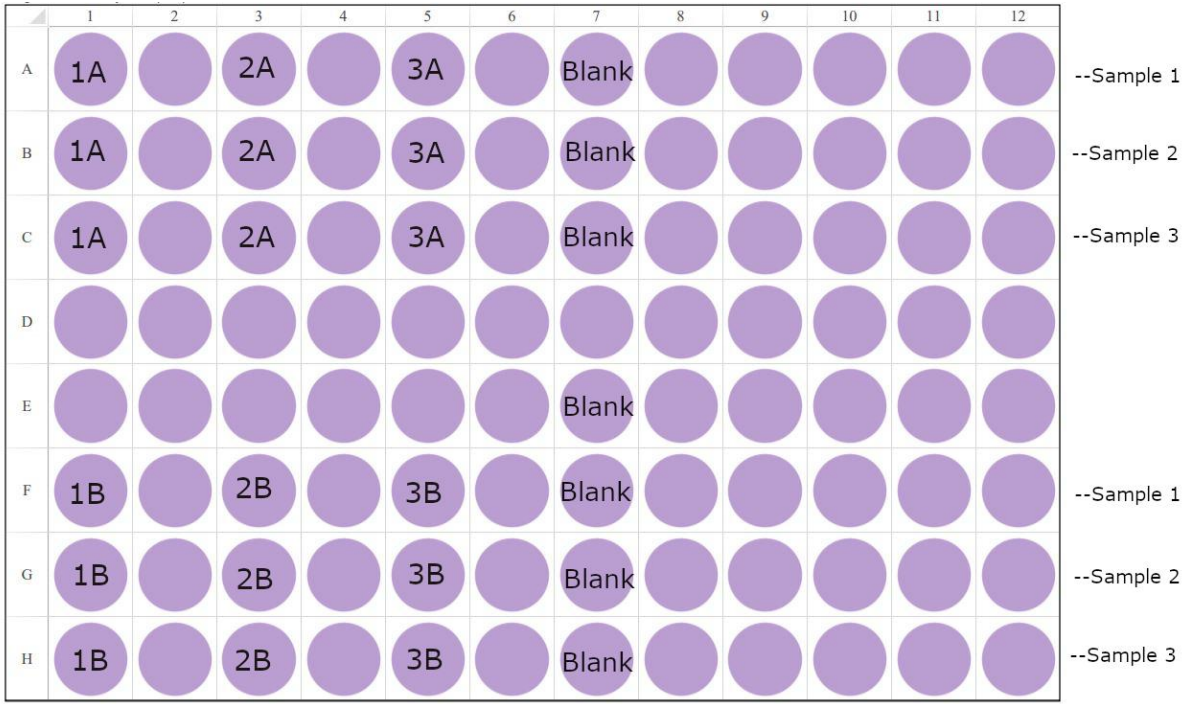
Week 2

AIM: To analyse the growth of fungi by its changing OD.

1. 09.07.2021:
    a. Slant PDA were made of pure culture samples.
    b. 3 different fungal species were taken:
        i. *Rhizopus oryzae*
        ii. *Aspergillus niger (*yet to be identified through sequencing, identified through morphology)
        iii. *Aspergillus versicolor*
    c. 2 slant culture of each sample has been made and kept in incubation of the cultures to grow at 28 degree celsius.
    d. Samples are marked as:
        i. 1A : *Aspergillus niger*
        ii. 1B : *Aspergillus niger*
        iii. 2A : *Aspergillus versicolor*
        iv. 2B : *Aspergillus versicolor*
        v. 3A : *Rhizopus oryzae*
        vi. 3B : *Rhizopus oryzae*

Week 3

1. 15.07.2021:
    a. Spore suspension was made using PBS and 0.1% Tween 20 was added to the suspension to prevent clubbing of spores.
    b. Counting of spores using Hemocytometer.
    c. The spores suspensions are stored at 4 degree celsius refrigerator.
2. 16.07.2021:
    a. Spores are diluted to 8*10^4 spores/mL and 16*10^4 spores/mL using PBS.
    b. The 96-well plate is set according to the plate design attached below and readings are taken in TECAN plate reader after 6hr interval at 605nm.

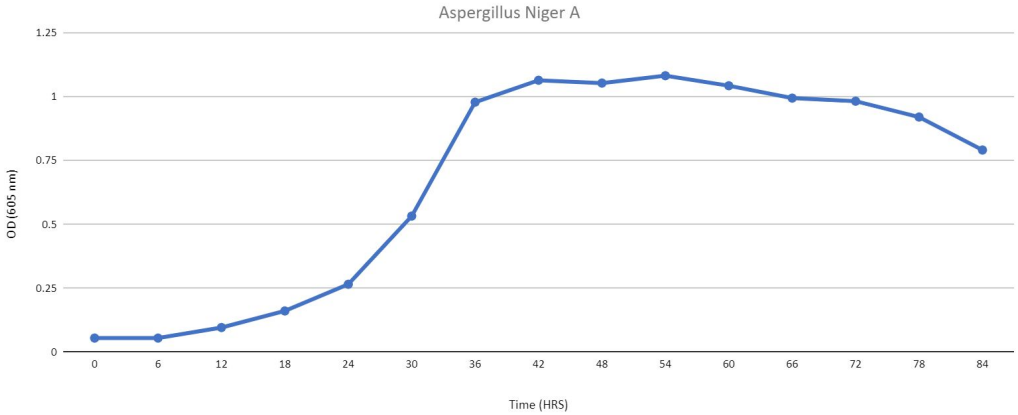


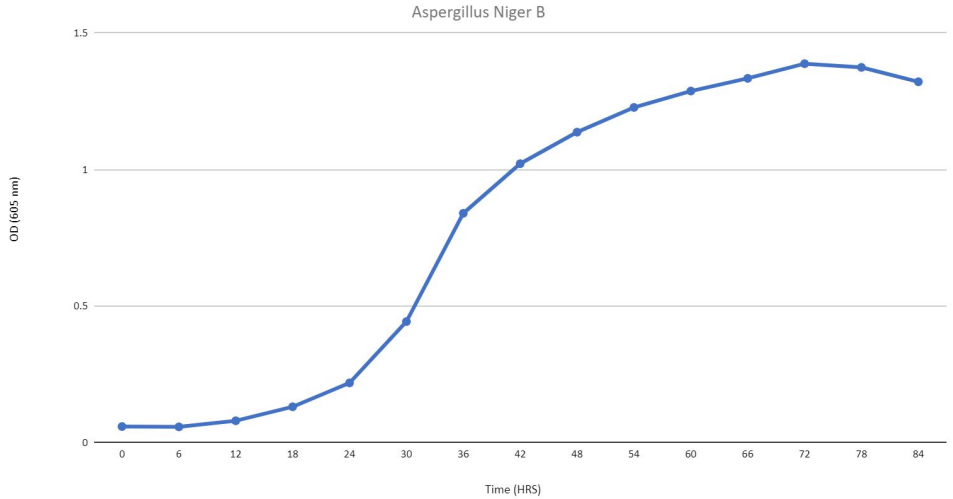
1. 1A : *Aspergillus niger*
2. 1B : *Aspergillus niger*
3. 2A : *Aspergillus versicolor*
4. 2B : *Aspergillus versicolor*
5. 3A : *Rhizopus oryzae*
6. 3B : *Rhizopus oryzae*

Composition in wells with samples:
100µL PDB + 100µL spore suspension
Blank: 100µL PDB + 100µL PBS

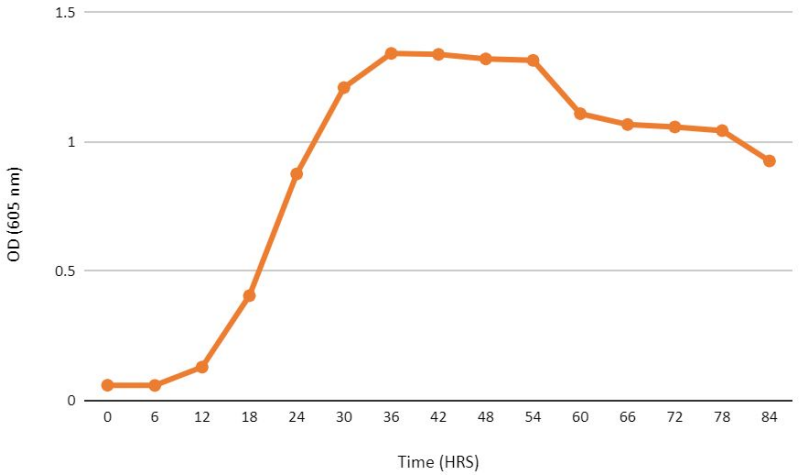
Note: All wells with spores have conc 2000 spores/mL

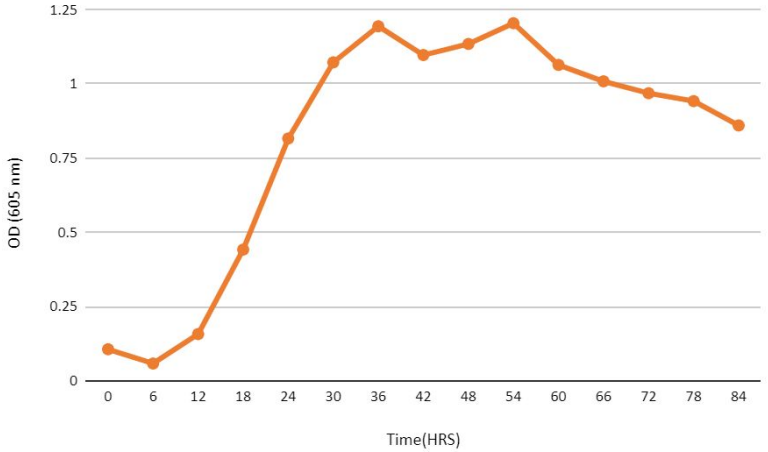
Aspergillus niger



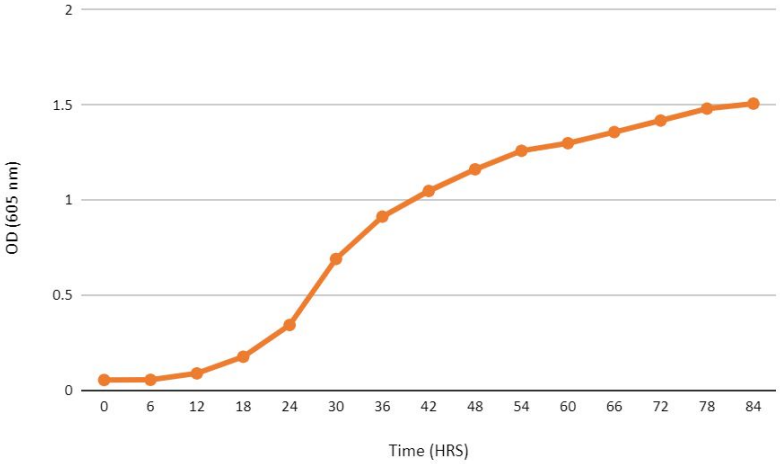


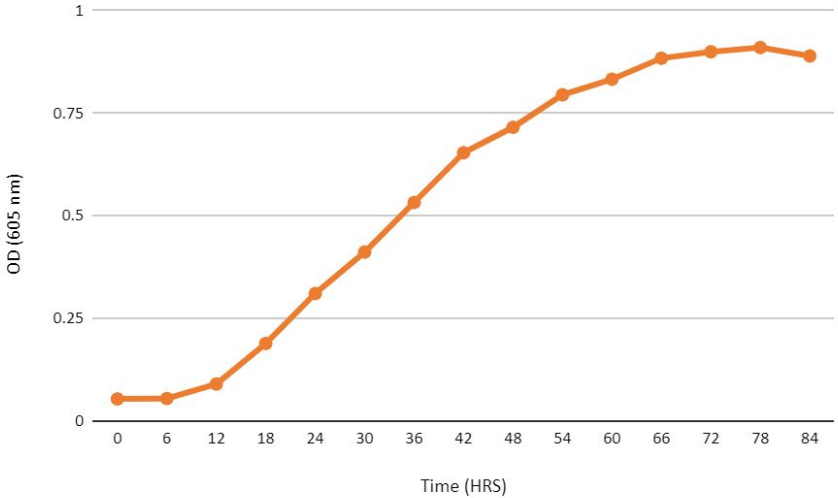
Rhizopus oryzae





Aspergillus versicolor





IMPORTANT NOTE

- Create a humid chamber, so that the solution in the wells is not evaporated fast.
- For creating a humid chamber, put some beakers with water in the incubator.
- The PDB to be made is to have a concentration of 2x as another 100ul of some other solvent is being added, decreasing the concentration of PDB to 0.5x if used 1x initially.

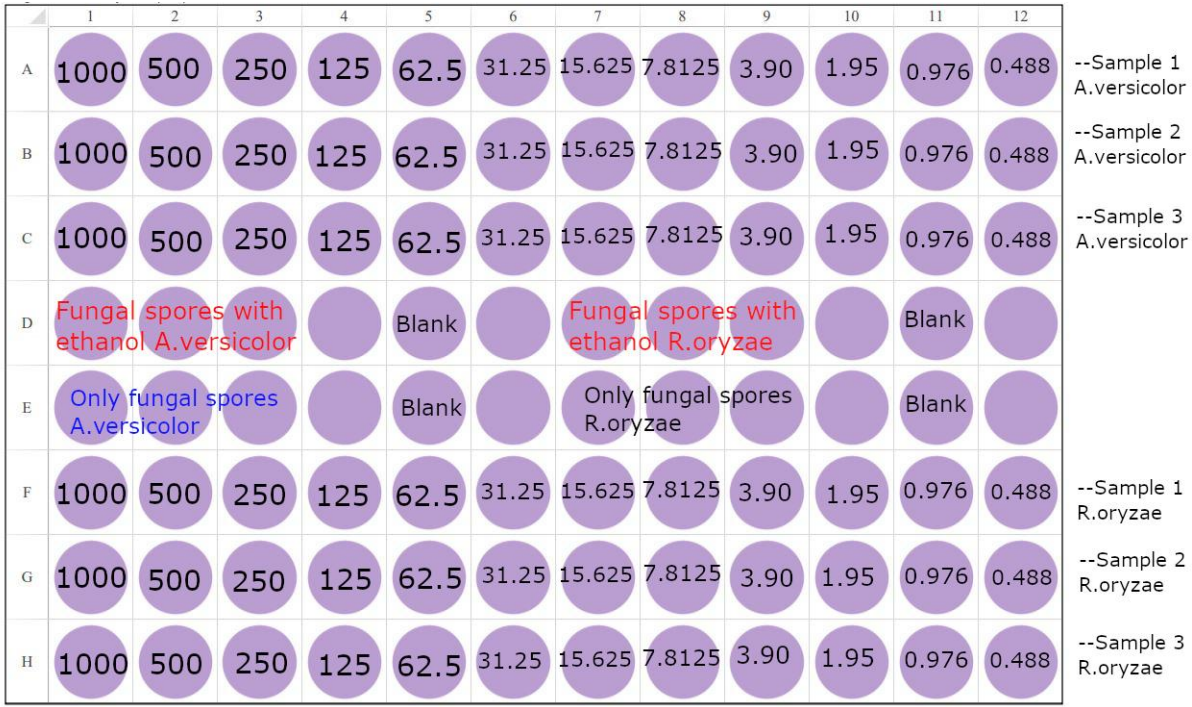
Week 5

AIM: To calculate the IC_{50} of Nipagin for 2 fungal species.

1. 23.07.2021:
   a. Spores were diluted to 4*10^4 spores/mL.
2. 24.07.2021:
   a. 12 dilutions of Nipagen were prepared in 100% ethanol

Tube no.	1	2	3	4	5	6	7	8	9	10	11	12
Conc(µg/mL)	1000	500	250	125	62.5	31.25	16.625	7.8125	3.90625	1.953125	0.9765625	0.488

   b. The 96-well plate is set according to the plate design attached below and readings are taken in TECAN plate reader after 6hr interval at 605nm.



D1,D2,D3 composition:
100μL PDB+ 50μL ethanol(100%)+50μL spore suspension(A.versicolor)

D7,D8,D9 composition:
100μL PDB+ 50μL ethanol(100%)+50μL spore suspension(R.oryzae)

E1,E2,E3 composition:
100μL PDB+ 100μL spore suspension(A.versicolor)

E7,E8,E9 composition:
100μL PDB+ 100μL spore suspension(R.oryzae)

In all other well in row A,B,C,F,G,H, composition:
100μL PDB+ 50μL Nipagin(in ethanol 100%)(conc in μg/mL) +50μL spore suspension
Blank: 100μL PDB + 100μL PBS

Note: All wells with spores have conc 2000 spores/mL

AUGUST

Week 1

1. 5/08/2021 : Fungal cultures were made in slant PDA
    a. *Aspergillus niger*
    b. *Rhizopus oryzae*

Important note :

1. F.solani isn't considered for this experiment as the risk group of the fungus is uncertain.
2. A.niger isn't considered for this experiment as it has been been identified through sanger-sequencing and procure many spores which can lead to contamination in the plate to other fungi.

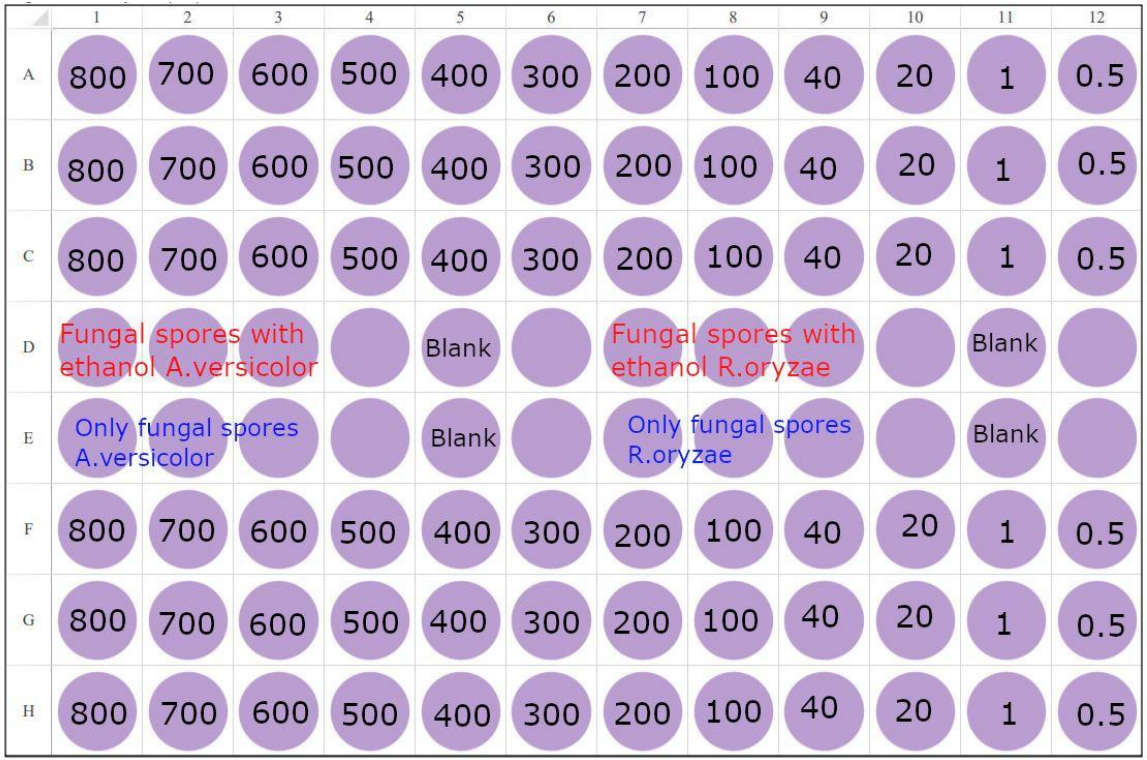
Week 2:

AIM: To calculate the IC_{50} of Nipagin for 2 fungal species.

1. 9/08/2021 :
    a. Spore suspension was made using 5mL PBS+5µL Tween 20.
    b. Spores were counted using a haemocytometer.
    c. Spores were diluted to 8*10^4 spores/mL and 4*10^4 spores/mL using PBS.
2. 10/08/2021:
    a. Making of 12 concentrations of nipagin in ethanol(100%)

Tube no.	1	2	3	4	5	6	7	8	9	10	11	12
Conc(µg/mL)	800	700	600	500	400	300	200	100	40	20	1	0.5

    b. The 96-well plate is set according to the plate design below and readings were taken after 6hr intervals in TECAN plate reader at 605nm.



D1,D2,D3 composition:
100μL PDB+ 50μL ethanol(100%)+50μL spore suspension(A.versicolor)

D7,D8,D9 composition:
100μL PDB+ 50μL ethanol(100%)+50μL spore suspension(R.oryzae)

E1,E2,E3 composition:
100μL PDB+ 100μL spore suspension(A.versicolor)

E7,E8,E9 composition:
100μL PDB+ 100μL spore suspension(R.oryzae)

In all other well in row A,B,C,F,G,H,
composition:
100μL PDB+ 50μL Nipagin(in ethanol 100%)(conc in μg/mL) +50μL spore suspension

Blank: 100μL PDB + 100μL PBS

Note: All wells with spores have conc 2000 spores/mL

1. 12/08/2021:
    a. Experiment dropped due to Interruption in the reading timings.
    b. The growth of fungus was non-uniform, more growth in higher conc of nipagin than the other.

Week 3

1. 15/08/2021:
    a. A new trial was initiated following the same plate design and using the same spore suspension and nipagin concentration as done on 9.08.2021-10.08.2021.
2. 21/08/2021:
    a. Experiment terminated at 2AM.
    b. Reason: There was fungal growth in the blank and non-uniform growth of both the fungi.

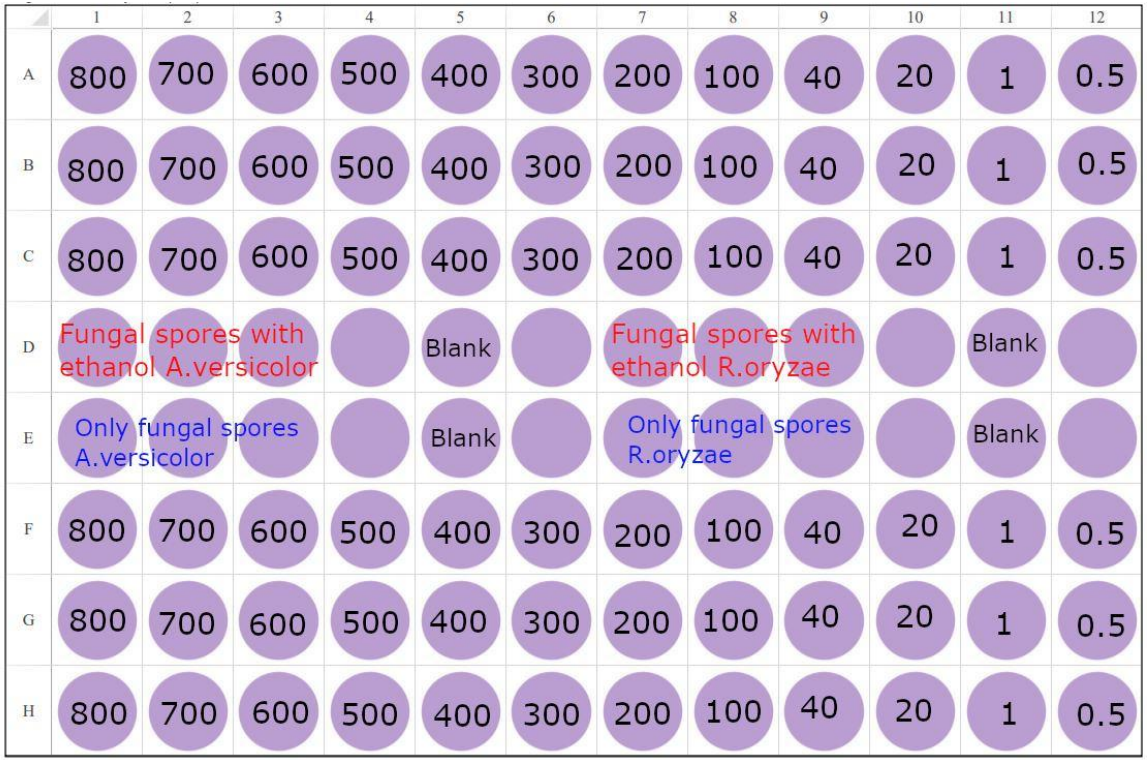
Week 4

AIM: To calculate the IC_{50} of Nipagin for 2 fungal species.

1. 27.08.2021:
    a. 2 fungal cultures were made in slant PDA.
        i. *Aspergillus versicolor*
        ii. *Rhizopus oryzae*
2. 30.08.2021:
    a. Dilutions of 12 concentrations of nipagin according to the table:

Tube no.	1	2	3	4	5	6	7	8	9	10	11	12
Conc(µg/mL)	800	700	600	500	400	300	200	100	40	20	1	0.5

    b. Making of spore suspension using PBS and Tween 20
    c. Counting of spores using Hemocytometer
3. 31.08.2021:
    a. Spores were diluted to 8*10^4 spores/mL and 4*10^4 spores/mL using PBS.
    b. Setting up of 96-well plate according to the design in next slide.
    c. 31.08.2021-8.09.2021: OD measured in TECAN plate reader at 605 nm.



D1,D2,D3 composition:
100μL PDB+ 50μL ethanol(100%)+50μL spore suspension(A.versicolor)

D7,D8,D9 composition:
100μL PDB+ 50μL ethanol(100%)+50μL spore suspension(R.oryzae)

E1,E2,E3 composition:
100μL PDB+ 100μL spore suspension(A.versicolor)

E7,E8,E9 composition:
100μL PDB+ 100μL spore suspension(R.oryzae)

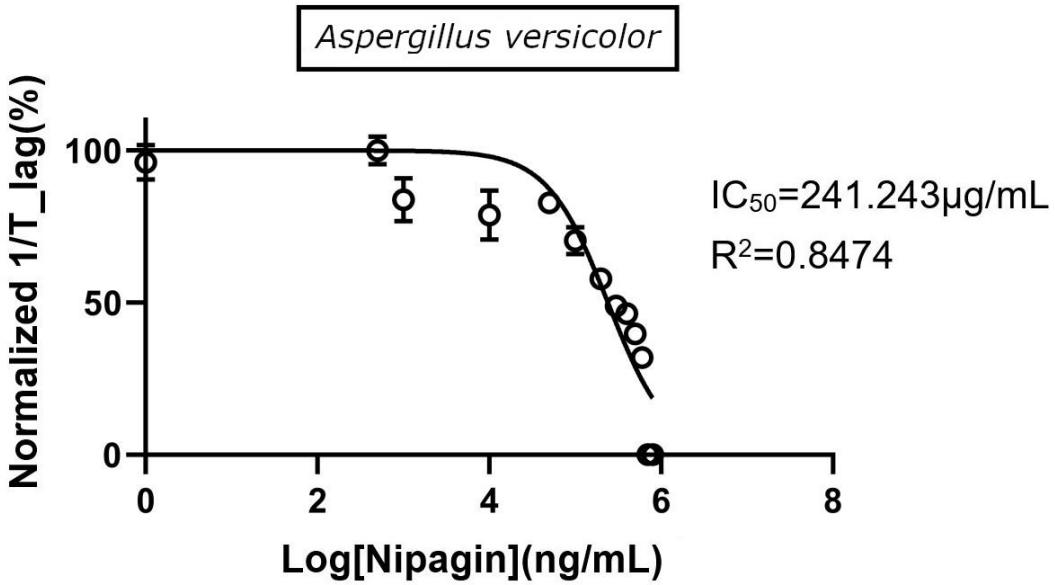
In all other well in row A,B,C,F,G,H,
composition:
100μL PDB+ 50μL Nipagin(in ethanol 100%)(conc in μg/mL) +50μL spore suspension

Blank: 100μL PDB + 100μL PBS

Note: All wells with spores have conc 2000 spores/mL

A.versicolor

conc.(ug/ml)	S1 (1/T_lag)	S2 (1/T_lag)
0	0.02225833018	0.02505650241
0.5	0.0235120958	0.02573856821
1	0.01889719736	0.0223914017
10	0.01741250218	0.02140869193
50	0.01992825827	0.02088118605
100	0.01621796951	0.01841959845
200	0.01448435689	0.01401345291
300	0.01190698738	0.0122037092
400	0.01107419712	0.01178133836
500	0.01012760786	0.009436891262
600	0.007613826709	0.008121761448
700	0	0
800	0	0



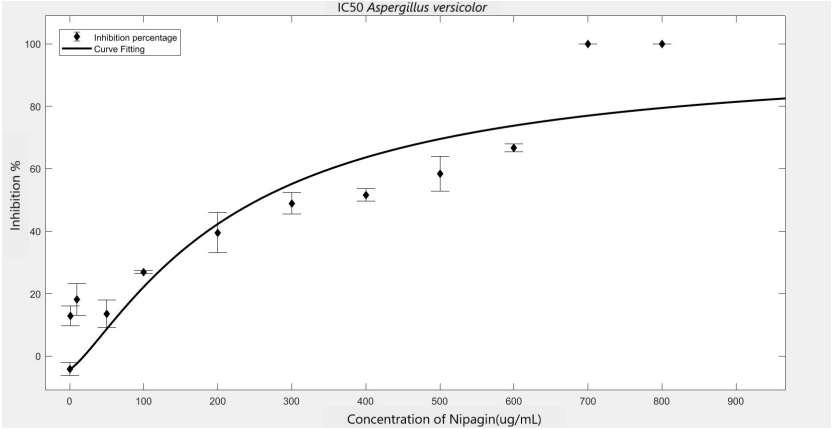


Fig: IC50 of Nipagin against *A.versicolor* using Method_1

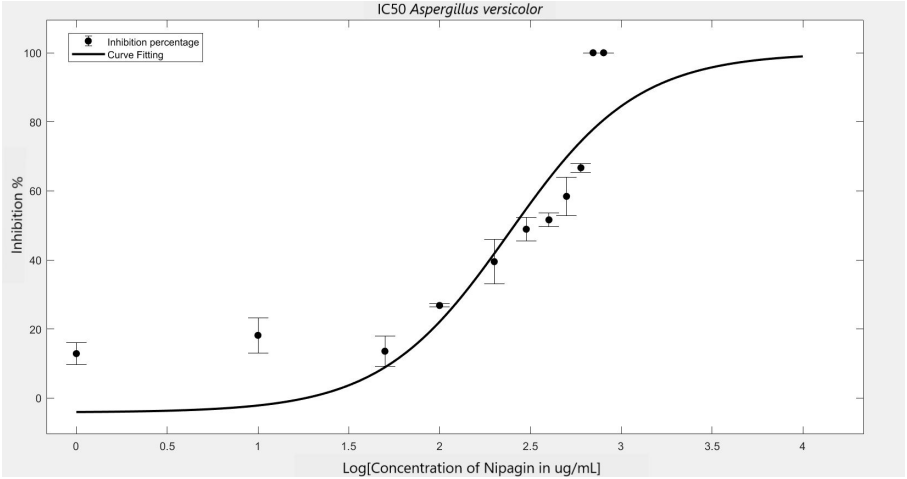


Fig: IC50 of Nipagin against *A.versicolor* using Method_2

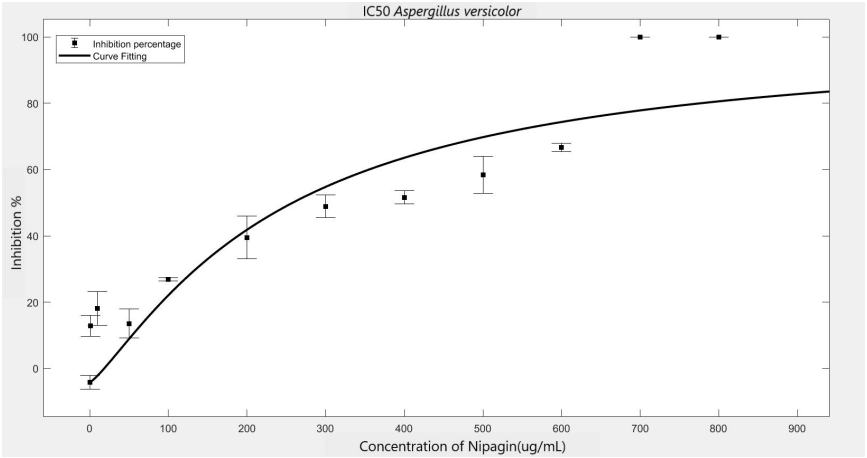
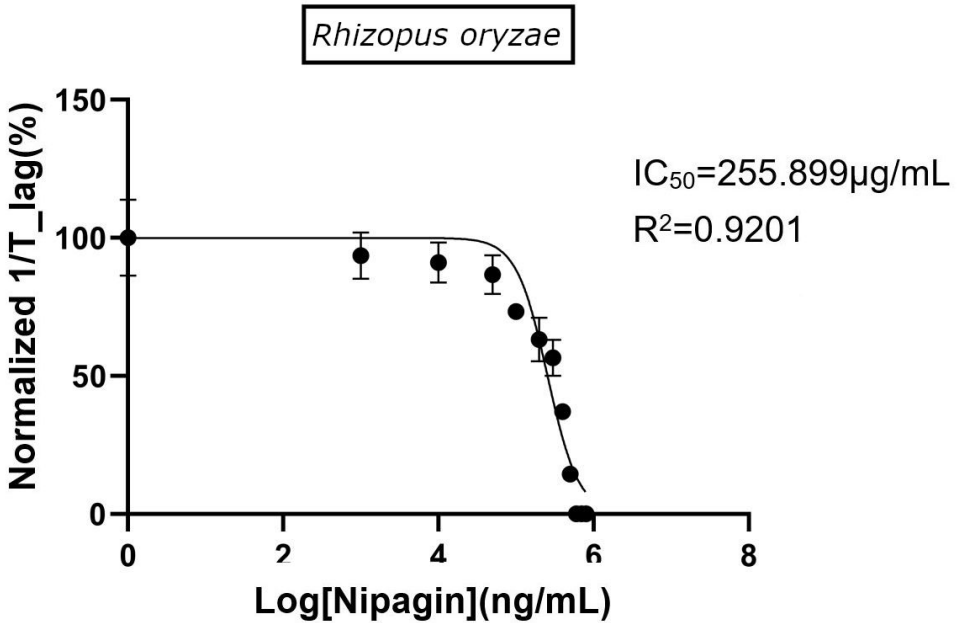


Fig: IC50 of Nipagin against *A.versicolor* using Method_3

Method used	Hill coefficient	IC50 (µg/mL)
Method 1	1.2785	223.534
Method 2	1.2308	241.9844
Method 3	1.2308	241.9797
Using GraphPad		241.243
	(R^2=0.8474)	

R.oryzae

Conc.(μg/ml)	1/T_lag(S1)	1/T_lag(S2)
0	0.04456327986	0.05422993492
1	0.04329004329	0.04918839154
10	0.04240396562	0.0475217412
50	0.04036538749	0.04530052367
100	0.03578636969	0.03660268517
200	0.02843874153	0.03397558514
300	0.02569373073	0.03021148036
400	0.01767830783	0.01888759614
500	0.007123521869	0.007123521869
600	0	0
700	0	0
800	0	0



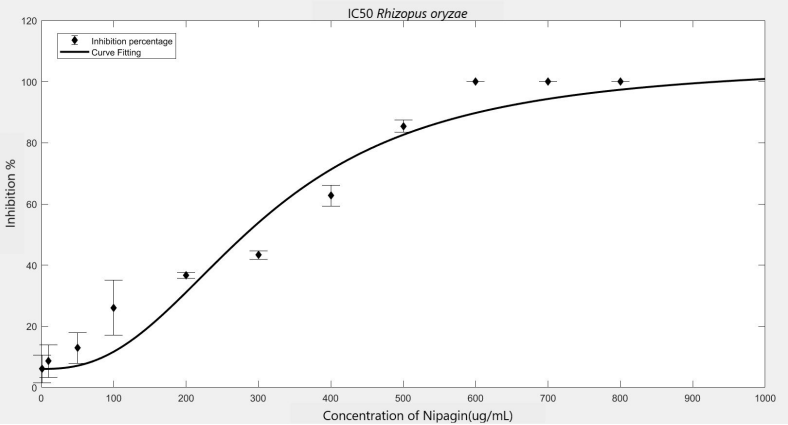


Fig: IC50 of Nipagin against *R.oryzae* using Method_1

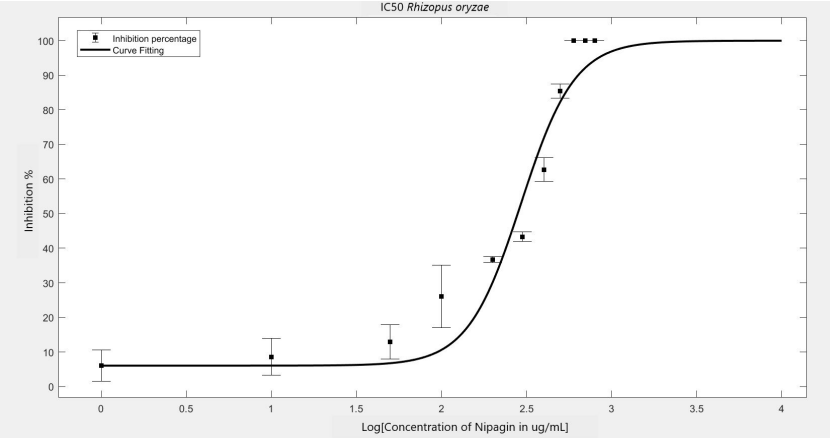


Fig: IC50 of Nipagin against *R.oryzae* using Method_2

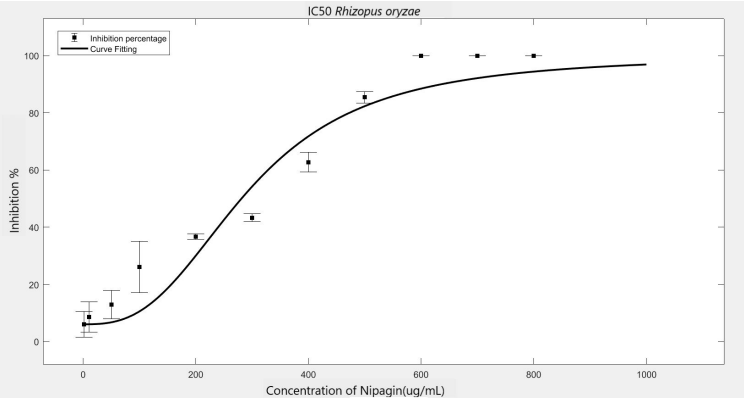
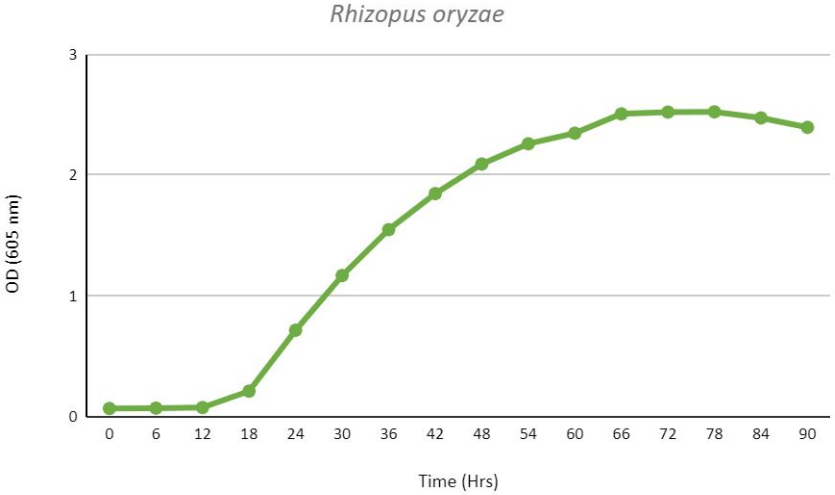
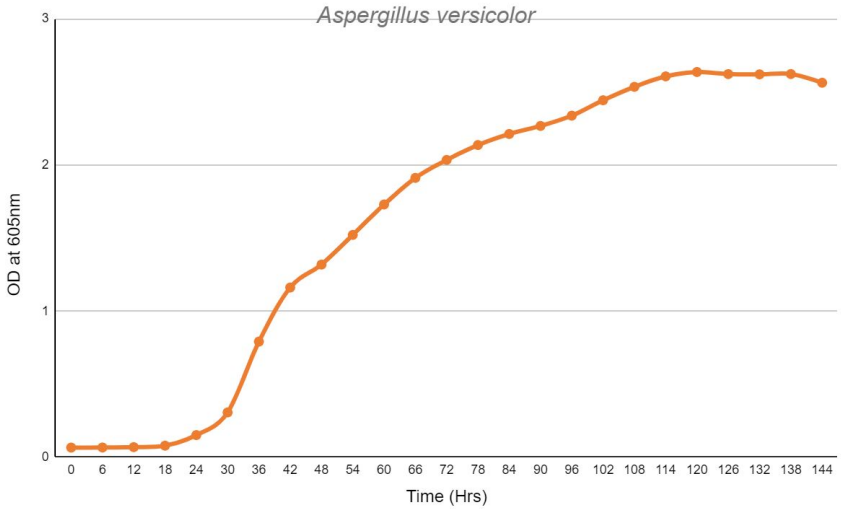


Fig: IC50 of Nipagin against *R.oryzae* using Method_3

Method used	Hill coefficient	IC50 (µg/mL)
Method 1	2.4848	2310.6738
Method 2	2.7604	294.2999
Method 3	2.7604	294.2999
Using GraphPad	-2.172	255.899
	(R^2=0.9202)	





100μL PDB(2X) + 100μL spore suspension(*R.oryzae*)

100μL PDB(2X) + 100μL spore suspension(*A.versicolor*)

	Growth rate (Absorbance/hr)	Lag time (hr)
Rhizopus oryzae	0.3145	27.69
Aspergillus versicolor	0.1701	42.72

SEPTEMBER

Week 4

AIM: To calculate the IC_{50} of the engineered chimeric chitinase for 2 fungal species.

25.09.2021: Fungal culture of Rhizopus oryzae was made in slant PDA.

Week 5

1. 27.09.2021: Spore suspension was made in 3mL PBS with 3µL of Tween 20 and stored at 4 degree celsius refrigerator.
2. 28.09.2021:
   a. No. of spores were counted using haemocytometer.
   b. Spores were diluted to 20*10^4 spores/mL, 8*10^4 spores/mL and 4*10^4 spores/mL using PBS.
   c. Dilutions of 12 concentrations of nipagin according to the table:

Tube no.	1	2	3	4	5	6	7	8	9	10	11	12
Conc (µg/mL)	800	700	600	500	400	300	200	100	40	20	1	0.5

C. Dilutions of 12 concentrations of Bacterial_chitinase_combo_2 using PBS according to the table:

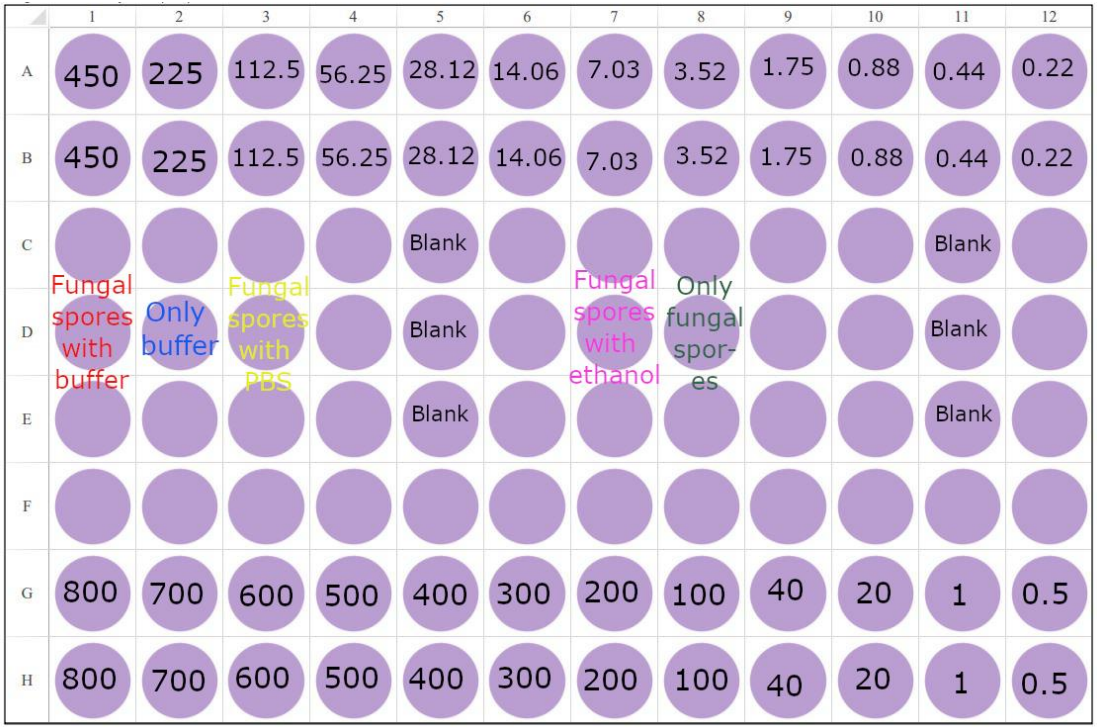
Tube no.	1	2	3	4	5	6	7	8	9	10	11	12
Conc(µg/mL)	450	225	112.5	56.25	28.125	14.0625	7.03125	3.15625	1.75	0.88	0.44	0.22

D. Setting up of 96-well plate according to the design in next slide.

E. Readings were taken at an interval of 6 hrs at 605nm.

The buffer that is used to store the protein has the following composition:

0.1%SDS , 10% glycerol, 1X PBS, 200 mM NaCl



C1, D1, E1: 100µL PDB+10µL spore suspension 90µL buffer
C2, D2, E2: 100µL PDB+100µL buffer
C3, D3, E3: 100µL PDB+10µL spore suspension 90µL PBS
C7, D7, E7: 100µL PDB+50µL spore suspension + 50µL ethanol(100%)
C8, D8, E8: 100µL PDB+100µL spore suspension.

Row A and B: 100µL PDB+10µL spore suspension+90µL protein
Row G and H: 100µL PDB+50µL spore suspension+50µL Nipagin(in 100%ethanol)(conc in µg/mL)
Blank: 100µL PDB+ 100µLPBS

Note: All wells with spores have conc 2000 spores/mL

Note:

1. The readings were stopped at 2AM on 1.10.2021 due to non-uniform readings and fungal growth in the blank.
2. Moreover, there was no significant antifungal activity shown by the protein.
3. Some of the wells turned pink in color(reasons yet unknown)