INDUCTION OF MUTANTS IN *Neurospora crassa* AND ITS GENETICAL AND BIOCHEMICAL INVESTIGATION

A DISSERTATION SUBMITTED FOR THE DEGREE OF

MASTER OF PHILOSOPHY

TO THE DEPARTMENT OF BOTANY

By

MAMTAZ BEGUM

B.Sc. (Hons), M.Sc.

DEPARTMENT OF BOTANY
UNIVERSITY OF DHAKA
DHAKA-1000, BANGLADESH

October, 2007

Dedicated
To
My Husband
(Md. Ismail Miah)

CERTIFICATE

I hereby certify that the research work embodying the results reported in this thesis entitled **"Induction of mutants in *Neurospora crassa* and its genetical and biochemical investigation".**

Submitted by Mamtaz Begum has been carried out under my guidance and suitable for submission in the form of a thesis for the partial fulfillment of the degree of Master of Philosophy in Botany, University of Dhaka.

Tahsina Rahim.

(Dr. Tahsina Rahim)
Professor and supervisor
Department of Botany
University of Dhaka.

DECLARATION

I here by declared that this thesis entitled **"Induction of mutants in *Neurospora crassa* and its genetical and biochemical investigation"** has been composed by myself and all the work has not been submitted anywhere for any academic degree.

October, 2007

Mamtaz Begum
Department of Botany
University of Dhaka
Dhaka- 1000, Bangladesh.

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This work presented here has been carried out in the Microbial Genetics Laboratory of the Department of Botany, University of Dhaka, under the sincere and careful guidance of my supervisor Dr. Tahsina Rahim, professor, Department of Botany, University of Dhaka.

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The Author

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LIST OF ABBREVIATIONS

Em	Emerson stock
Ema	Emerson stock mating type 'a'
EmA	Emerson stock mating type 'A'
VM	Vogel's minimal medium
VM^+	Supplemented Vogel's minimal medium
SM	Sorbose minimal medium
SM^+	Supplemented sorbose minimal medium
WCM	Westergaard's crossing medium
cm	centimeter
g	gram
mg	milligram
ml	millilitre
%	percentage
leu-3	leu-3 auxotroph
arg-5	arginine-5 auxotroph
trp-1	tryptophan-1 auxotroph
trp-4	tryptophan-4 auxotroph
lys-1	lysine-1 auxotroph
trp-2	tryptophan-2 auxotroph
arg-10	arginine-10 auxotroph
ro.	ropy
vg.	vigorus
con. band	conidial band
clf.	cauliflower
fl.	fluffy
fl. band	fluffy band
al.	albino
cr.	crescent

ABSTRACT

Neurospora crassa, the experimental material of this work is an excellent tool of genetical research. It was analyzed for spontaneous mutation. But it was found that there were no spontaneous mutants present in Ema, the stock of Microbial Genetics Research Laboratory, Department of Botany, University of Dhaka.

It was observed that the concentration of leaf extract of *Adhatoda zeylanica* Nees. controlled the radial growth of wild type *Neurospora crassa* (Ema).The result also showed that non-sterilizedconcentration was most effective than sterilized one.Non-sterilized A_0 concentration was the most effective and sterilized A_3 concentration was the least effective. Non-sterilized A_0 and A_1 concentration were chosen for treating the conidia of Ema for induction of mutation and five groups of mutants were found. These are ropy, cauliflower, crescent, checked and vigorous.

It was found that leaf extract of *Centella asiatica* (Linn.) Urban. possesses antifungal properties and it reduced the radial growth of *Neurospora crassa* as compared to the control.

The result also showed that sterilized leaf extract of *Centella asiatica* (Linn.) Urban. was less effective than non-sterilized one. Non-sterilized C_0 concentration was the most effective and sterilized C_3 concentration was the least effective. Non-sterilized C_0 and C_1

concentration were selected for treating the conidia of Ema for induction of mutation. Four groups of mutants were found from the treatment. These are plug, fluffy band, mat and checked.

It was also found that the concentration of fruit extract of *phyllanthus emblica* Linn. controlled the radial growth of *Neurospora crassa.*

The result also showed that non-sterilizedconcentration was more effective than sterilized one. Non-sterilized P_0 concentration was the most effective and sterilized P_3 concentration was the least effective. Non-sterilizedP_0 and P_2 were chosen for treating the conidia of Ema for induction of mutaion and six group of mutants were found. These are albino, checked, fluffy , conidial band, buff and ropy.

A good number of morphological mutants induced with leaf extract of *Adhatoda zeylanica* Nees., *Centella asiatic*a (Linn.) Urban and fruit extract of *phyllanthus emblica* Linn. were selected for further study. Clear variations were observed when comparative study of Ema and the selected mutants were undertaken. These variations were confirmed by the dry weight of mycelia, radial growth rate, linear growth rate, initiation of conidial germination etc.

The selected morphological mutants were crossed with Ema and EmA separately and their segregating ratio was studied. Mating types of some extract were also checked. The progenies showed about. 1: 1 ratio in their morphology and mating type. So it was concluded that the mutant characters have resulted due to mutation in nuclear gene.

Linkage of some mutants, ropy ($A_0$307), conidial band ($P_0$729), checked ($A_0$380), fluffy (P_2 945), vigorous ($A_1$550) were determined. Distances of the mutants with the respective markers were calculated and given below. Mutant ropy ($A_0$307) was found to be linked with leu-3 (R156)A of linkage group 1 and gave a distance of 34.88 centimorgan. Mutant conidial band ($P_0$729) was found to be linked with trp-2 (75001) A of linkage group VI and gave a distance of 29.41 centimoragn. Mutant checked ($A_0$380) was found to be linked with trp-4 (Y 2198)A of linkage group IV and gave a distance of 32. 38 centimorgan. Mutant fluffy (P_2 945) was found to be linked with trp-4 (Y2198)A of linkage group IV and gave a distance of 32.29 centimorgan . Mutant vigorous ($A_1$550) was found to be linked with trp-1(10575)A of linkage group III and gave a distance of 18.75-centimorgan.

INTRODUCTION

1. INTRODUCTION

Neurospora crassa is a filamentous fungus that belongs to theClass-Ascomycetes, Subclass-Pyrenomycitadae, Order- Xylariales. Family-Sordariaceae.

Neurospora crassa is heterothallic and as a result there are two mating types called 'A' and 'a'. It has distinct male and female sexual structures, both borne on the same hermaphroditic mycelium. The female organ is the protoperithecium, which consists of a coilled cell, the ascogonium extending into a receptive hypha and enclosed by a small knot of hypha. The male function of the fertilization of the ascogonium by strain of the other mating type can be carried out (most commonly) by a conidium or by a suitably placed hypha.

Neurospora species is almost unique. They also differ from most of the plants and animals in their life cycles and biology. An acquaintance with those characteristics feature is therefore, a prerequisite for understanding their genetics.

More over *Neurospora* is a eukaryote, its genetics can be related to those organisms whose study had laid down the foundation of genetics.

It can easily grow on minimal medium, a mixture of mineral salts, in water plus a carbon source (sucrose) and the vitamin biotin (Beadle

and Tatum 1941, Anonymous 1966). A much shorter time is required to obtain a number of generations of *Neurospora* than of higher plants and animals. In addition, it can be grown in test tubes, require less space and less expensive than most higher plants and animals. Because of it's short life cycle and capacity to grow on minimal medium help to perform extensive work with in a period of short time. so, the author preferred the species *Neurospora crassa* as experimental material for genetical study .

Beadle and Tatum (1941) first used the *Neurospora crassa* in genetical and at the same time for biochemical study. *Neurospora* has provided geneticists with novel genetic systems and with the opportunity of studying particular problems with greater case and precision than is possible in other organism. And as a result the genetics of *Neurospora crassa* have been studied fairly intensively during the recent years.

(Akins and Lambowitz 1985. Aranson *et al* 1994: Atkinson *et al* 1995; Bruchez *et al* 1993; Case *et al* 1992 : kappor *et al* 1995; Mc Donald 1995; Crawford *et al* 1992; Fossa *et al* 1995; Kuldau *et al* 1993; Pall *et al* 1993; Yang *et al* 1993; Yassin and Wheals 1992).

Presently scientists are interested to evaluate the antifungal activities of plant extract against plant pathogenic fungi. Haque and Shamsi (1996) observed that leaf extract of Neem has antifungal properties and it decreased the radial growth of *Macrophomina phaseolina*. In most of

the treatment sporulation of the fungus was reduced, radial growth decreased and colony become compressed as compared to the control. Antifungal properties of the plant extract are responsible for inhabition of growth of the fungus (Haque and Shamsi 1997).

What are the mutagens with which mutation could be induced or what is the molecular mechanism of mutation has been the subject of the investigation during the past years (Ahmad *et al* 1977; Ahmad and Catcheside 1960; Arganoza *et al* 1994; Beadle and Tatum 1945; DeSerres, F.J. 1994a; DeSerres and Malling 1995; Santosa and Kuwana 1992; Stephens *et al* 1993;. Velanji *et al* 1994; and Yu *et al* 1993).

Extract of the leaf of *Adhotoda zeylanica* Nees. was investigated for antimicrobial, analgesic, antispasmodic and hypoglycaemic activities. *Adhatoda zeylanica* Nees. is valued in medicine as a expectorant and stimulant to the gastrointestinal troct. Leaf of extract of *Adhatoda zeylanica* Nees. is used in various chest ailments including cough asthma, bronchitis, pneumonia and phthisis. Alcoholic extract of leaves in hypotensive. It is also used in ague.

Amloki whose botanical name is *Phyllanthus emblica* Linn. belonging to the family Euphorbiaceae. Fruit is a rich natural source of vitamin C. Extract of *Phylanthus emblica* Linn. In used as a diuretic, refrigerant, carminative, stomachic, laxative, antacid and tonic;

promotes children's resistance to cold and cough. It is also used in vomiting and burning urination and as a hair tonic.

The author here tested the mutagenic properties of leaf extract of *Adhatoda zeylanica* Nees., *Centella asiatica* (linn.) Urban. and fruit extract of *Phyllanthus emblica* Linn. These extract by applying it on the growth of *N. crassa*. Different concentrations of leaf and fruit extract were used for this investigation. Conidia of Ema were treated with leaf and fruit extract. The author tried to find out the minimum dosages that cause mutation. It was examined that leaf and fruit extract has any mutagenic properties at which dosage of concentration or not.

Now a days a good number or insecticides, weedicides and fungicides are used as mutagenesis for mutation and biological control (Bignami *et al* (1977), Velazquez *et al* (1987), Barrueco *et al* (1988), Eiche *et al* (1990), Flessel *et al* (1993), Mutero *et al* (1994), Nurzhanova *et al* (1994), Jasieniuk *et al* (1994), Piatti *et al* (1994), Bianchi *et al* (1994), Mutero *et al* (1994), Stehrer-Schmid and Wolf (1995), Kumar *et al* (1995), Pluth *et al* (1996)).

Mutation is a sudden hereditary change found in all living organism. It readily accounts for all variations and is the building block of evolution. An understanding of the mechanism of mutation is essential to the study of genetics.

Linkage was first demonstrated in *Neurospora crassa* by lindegren (1933), Subsequently he published linkage maps of linkage group I and II (Lindegren and Lindegren 1939)

Therefore, the author made a plan to determine –

i) If leaf extract of *Adhatoda zeylanica* Nees, *Centella asiatica* (Linn.) Urban and fruit extract of ***phyllanthus emblica*** Linn, can produce new mutants.

ii) Whether the obtained mutants resulted due to the mutation in nuclear gene by crossing them with Ema and EmA separately and by studying their segregation.

iii) The linkage of some selected mutants by crossing them with marker reprsentatives of all the seven linkage groups.

iv) To prepare genetic map of some selected induced mutants.

MATERIALS

2. MATERIALS

The experimental material was *Neurospora crassa*. It is an ascomycetes fungus. This material was kindly supplied by prof. T.I.M.A. Mozmader of the Department of Botany, University of Dhaka.

Neurospora crassa possesses interwined segment branched hyphal filaments some 5 μ or less in diameter. Each hyphal segment contains haploid nuclei with seven chromosomes. It exists as a soil saprophyte, grows on sugar-cane bagasse, carbon, vegetation or in bakeries. It is known as the "red bread mold" which is also called "Plant *Drosophila*" and grows in tropical or subtropical regions (Shear and Dodge, 1927).

The reproductive system of *N. crassa* contains both asexual and sexual spores. Asexual spores are called conidia and sexual spores are called ascospores. Sexual fruiting bodies which are called perithecia, produced by the contact of hyphae of two opposite mating types known as "EmA" and "Ema" strains when grown on a suitable agar crossing medium (Shear and Dodge, 1927. Westergaard and Mitchell, 1947, Anonymous 1966).

Culture Emerson "a" (Ema) (5297), Emerson "A" (EmA) (5296) and markers were used for the investigation. Ema was used for treating conidia with mutagens and for comparing growth of mutants. Both

"Ema" and "EmA" were used for checking mating types of mutants and extract and the markers were used for the study of linkage.

The markers and wild types were received from Fungal Genetics Stock Center Department of Microbiology, University of Kansas Medical Ceneter, U.S.A.

The following strains of *Neurospora crassa* were kindly supplied by professor Dr.T.I.M.A. Mozmader, from genetics stock, Department of Botany , University of Dhaka.

2.1 WILD TYPES

a) Emerson 'A' (EmA) (5296)

b) Emerson 'a' (Em'a') (5297)

2.2. STANDARD MARKERS

Name of the marker	No of the allele	Mating types	Position of the gene	Linkage group
leu-3	R156	A	IL	I
arg-5	27947	A	IIR	II
trp-1	10575	A	IIIR	III
trp-4	Y2198	A	IVR	IV
lys-1	33933	A	VL	V
trp-2	75001	A	VIR	VI
arg-10	B 317	A	VIIR	VII

2.3 MUTAGEN

Plants extract

a) Leaf extract of *Adhatoda zeylanica* Nees.

Scientific name : *Adhatoda zeylanica* Nees.

Bengali name : Basak

English name: Malabar nut

Family : Acanthaceae

Useful part : Leaf

b) Leaf extract of *Centella asiatica* (Linn.) Urban

Scientific name : *Centella asiatica* (Linn.) Urban

Bengali name : Thankuni

English name: Indian penny wort

Family : Apiaceae

Useful part : Leaf

c) Fruit extract of *Phyllanthus emblica* Linn.

Scientific name : *Phyllanthus emblica* Linn.

Bengali name : Amloki

English name: Indian goose berry

Family : Euphorbiaceae

Useful part : fruit

2.4 APPARATUS AND GLASS WARES

Apparatus and glass wares which were used for the present research works, are as follows.

a) Apparatus

1) Autoclave

2) Centrifuge

3) Refrigerator

4) Electric balance

5) Incubator

6) Inculation chamber

7) P^H meter

8) Shaker Gy Rotary

b) **Glasswares**

No.	Name	Made	Size	Purpose
1	Test tubes	Pyrex	0.5″×2″	Isolating tubes
2	Test tubes	Pyrex	0.5″×3″	Subculturing tubes
3	Test tubes	Pyrex	5/8″×5″	Stock tubes
4	Test tubes	Pyrex	1″×6″	Crossing tubes
5	Test tubes	Pyrex	1″×8″	Radiation tubes
6	Test tubes	Pyrex	0.5″×15″	Growth race tubes
7	Petridishes	Pyrex	1.5″ dia	Irradiation
8	Petridishes	Pyrex	3″ dia	Media
9	Flasks (Erlenmyer)	Pyrex	100ml	Media and water etc
10	Flasks (Erlenmyer)	Pyrex	250ml	Media and water etc
11	Flasks (Erlenmyer)	Pyrex	500ml	Media and water etc
12	Flasks (Erlenmyer)	Pyrex	1000ml	Media and water etc

2.5 CHEMICALS USED FOR THE RESEARCH WORK

a) General

1) Agar (Bacto)
2) Ammonium nitrate (NH_4NO_3)
3) Ammonium sulphate (NH_4SO_4)
4) Biotin ($C_{10}H_{16}N_2O_3S$)
5) Boric acid (H_3BO_4) anhydride
6) Calcium chloride ($CaCl_2$. 6 H_2 O)
7) Chloroform ($CHCl_3$)
8) Citric acid [C (OH) (COOH) $(CH_2COOH)_2$. H_2O]
9) Copper sulphate ($CuSO_4$. $7H_2O$)
10) Ferrous sulphate ($FeSO_4$. $7H_2O$)
11) Hydrochloric acid (HCl)
12) Magnesium chloride ($MgCl_2$)
13) Magnesium sulphate ($MgSO_4$.$7H_2O$)
14) Manganese sulphate ($MnSO_4$.$7H_2O$)
15) Methanol (CH_3OH)
16) Potassium nitrate (KNO_3)
17) Potassium di- hydrogen phosphate (KH_2PO_4)
18) Sodium chloride (NaCl)
19) Sodium citrate ($NaH_2C_6H_5O_7 2H_2O$)
20) Sodium molybdate (Na_2MoO_4 . $2H_2O$)
21) Sorbose [OCH_2 $(CHOH)_3$]
22) Sucrose ($C_{12}H_{22}O_{11}$)
23) Zinc sulphate ($ZnSO_4$.$7H_2O$)

b) Amino-acid Supplement

1. Adenine
2. Arginine
3. Histidine
4. Isoleucine and valine
5. Lysine
6. Pyrimidine
7. Leucine
8. Tryptophan

c) Special Reagent

1. 0.0 5M phosphate buffer solution
2. 0.4M Sodium thiosulphate solution
3. Digestion mixture
4. 2% boric acid solution
5. 50% sodium hydroxide solution
6. Tuhhiroh reagent
7. Phenolphthalien aline

d) Stains

1. Cotton blue
2. Acridine orange.

MEDIA

3. MEDIA

3.1 STOCK SOLUTIONS NEEDED FOR DIFFERENT MEDIA.

For Culturing *N. crassa*, various stock solutions were needed for the preparation of media.

To prepare a stock solution, the chemicals were added one after another and the addition of the next one was followed by the complete dissolution of the previous one. 1000 ml conical flask was used to keep the stock solution . All the stock solution were stored in the refrigerator.

3.1.1 Composition Of Trace Elements Solution Per 100 ml

1. Distilled water	95 ml
2. Citric acid C(OH) (COOH)(CH_2COOH) H_2O	5g
3. Zinc sulphate ($ZnSO_4$.$7H_2O$)	5g
4. Ferrous sulphate ($FeSO_4$. $6H_2O$)	1.0g
5. Copper sulphate ($CuSO_4$. $5H_2O$)	0.25g
6. Manganese sulphate($MnSO_4$. H_20)	0.65
7. Boric acid anhydrous (H_3BO_4)	0.05g
8. Sodium molybdate ($NaMoO_4$. $2H_2O$)	
9. 9. Chlorform ($CHCl_3$)	1 ml

The solution is needed for the preparation of volegl's stock solution.

3.1.2 VOGEL'S STOCK SOLUTION

Vogel's stock solution was essential for the preparation of Vogel's minimal medium and sorbose minimal medium. 40 ml Vogel's stock

solution was required for 1000ml medium. The stock solution was kept in a refrigerator.

Composition Of Vogel's Stock Solution Per Liter.

1	Distilled water	770 ml
2	Sodium citrate	63.5g
3	Potassium dihydrogen phosphate (KH_2PO_4)	125g
4	Ammonium Nitrate (NH_4NO_3)	50g
5	Magnesium sulphate ($MgSO_4 . 7H_2O$)	5g
6	Calcium chloride ($CaCl_2. 6H_2O$)	3.75g
7	Trace elements solution	2.5ml
8	Biotin solution	1.25g
9	chloroform $CHCl_3$	2ml

1000 ml conical flask was used for keeping the stock solution. Calcium chloride was added first to the distilled water, then other chemicals were added one after another.

3.1.3 Composition Of Biotin Solution Per 100ml

1. Distilled water 100 ml
2. Biotin 10mg

Biotin was used for the preparation of Vogel's stock solution and for Westergaard's stock solution .

3.1.4 WESTERGARD'S STOCK SOLUTION

This solution was essential for the preparation of westergaard's crossing medium.250 ml Westergaard's stock solution was required for 1000 ml medium .

Composition of Wsestergaard's stock solution per liter.

1	Distilled water	1000 ml
2	Potassium nitrate (KNO_3)	4g
3	Potassium dihydrogen phosphate (KH_2PO_4)	4g
4	Magnesium sulphate ($MgSO_4 . 7H_2O$)	4.1g
5	Calcium chloride ($CaC_2 . 6 H_2O$)	0.8g
6	Sodium chloride (NaCl)	0.4g
7	Biotin solution	1.5m
8	Chloroform ($CHCl_3$)	2ml

3.1.5 STOCK SOLUTION OF AMINO-ACIDS

For the preparation of supplemented medium. Various amino-acid were added withVM medium.100 mg of amino-acid was dissolved in 100 ml of sterilized distilled water and 100 ml of amino-acid solution was used for 1000 ml medium.

Composition of amino-acid solutions

1. Distilled water 500 ml
2. Amino-acids 500mg

The stock solution was named after the amino-acidused. For example, leucine solution, arginine solution, tryptophan solution etc.

3.2 MEDIUM FOR CULTURING AND MAINTAINING STOCK

Neurospora crassa grown on a suitable mixture of mineral salts in water plus a carbon source (sucrose) and the vitamin biotin. The nutrition of *N. crassa* is very simple (Beadle and Tatum, 1945 Anonymous, 1966).Various medium were used for the growth and reproductionof *N. crassa.* Vogels minimal or Vogels supplemented medium was used for the maintenance of cultures, counting of spores and study of linear growth (Vogel 1956). Vogel's minimal liquid medium was used for the study of mycelial growth. Sorbose minimal or sorbose supplemented medium for single colony isolation and single spore isolation. Westergaasrd's crossing medium and Suyama's crossing medium were used for crossing to find out the mating types of mutants. All media were sterilized in the autoclave at 120^0C under 15 lb pressure for 20 minutes.

3.2.1 VOGEL'S MINIMAL MEDIUM

The medium was used for culturing *Neurospora cras*sa in the test tubes. It also used for growing and counting spores in the petriplate.Amino-acid supplements were used in culturing medium to the specific need of different mutants especially for biochemical mutants and the markers. The supplemented media were named after the amino-acid used; e.g. leucine medium, tryptophan medium, lysine medium etc.

2-4 ml of medium was taken in a small test tubes (3″) for maintaining cultures of mutant 8-10ml of medium was taken in a large test tubes (6″) for plating and isolating growing spores.

Biochemical mutants and wild types were easily recognized by testing them on Vogel's minimal medium (non-supplemented) plates. The growing ones were the wild types and the non growing (germinating) ones were the biochemical mutants.

Composition of Vogel's minimal medium (VM) Per Liter For Culturing.

1	Distilled water	960ml
2	Vogel's stock solution	40ml
3	Sucrose	20g
4.	Agar	15g.

VM with 1.5% agar was used for sub culturing and heterocaryon tests. In case of plating 2% of agar was used 100 mg of required amino-acid was used for supplemented VM per liter.

3.3 MEDIUM USED FOR PLATING

3.3.1 Sorbose Minimal Medium

Supplemented Sorbose medium as well as non-supplemented Sorbose medium were used for plating. This medium was used for plating as it had the quality to restrict the growth of the colonies.

The rapid linear growth rate of *Neurospora crassa* was a serious drawback when individual colonies and single spore needed to be isolated . The problem was circumvented by the finding that when certain chemicals, notably the ketohexose sorbose had been added to the culture media a restricted colonial growth habit results. The importance of the ratio between other carbon sources and sorbose has been investigated. (Brockman and De serres, 1963, Pittenger 1964).

This medium is very helpful in conidial isolation where the single conidial growth was restricted. This medium was also used as minimal test for wild type and mutant.

Composition of Sorbose Minimal Medium Per Liter

1.	Distilled water	960ml
2.	Vogel's stock solution	40ml
3.	Sucrose	1g
4.	Agar	20g
5.	Sorbose	5g

Sorbose was added after boiling all other constituents to avoid caramelization. For plating 15 ml of the medium was poured into each tube.

3.4 VOGEL'S MINIMAL LIQUID MEDIUM

VM liquid medium was used for obtaining mycelial growth and for heterocaryosis.

Composition of Vogel's Minimal Liquid Medium Per Liter

1.	Distilled water	960ml
2.	Vogels stock solution	40ml
3.	Sucrose	20g

The medium was supplemented with some selected amino-acids where necessary. To determine the mycelial growth of organism VM liquid media was used.

3.5. MEDIUM USED TO DETERMINE LINEAR GROWTH

This medium was used to determine the linear growth and growth rate of the organism.

Composition of Medium for Linear growth per liter

1.	Distilled water	960ml
2.	Vogel's stock solution	40ml
3.	Agar	40g
4.	Sucrose	20g

The medium was supplemented with amino-acids where necessary.

3.6 MEDIUM USED FOR CROSSING

3.6.1 WESTERGAARD'S CROSSING MEDIUM

This medium was used for crossing; it restricts the vegetative growth but enhances sexual reproduction. It is also used to determine the mating types of the mutants and extract and for the study of linkage(Westergaard and Mitchel, 1947).

Composition of Westergaard's Crossing Medium

1.	Westergaard's stock solution	250ml
2.	Distilled water	750ml
3.	Sucrose	20g
3.	Agar	20g

3.6.2 SUYAMA'S CROSSING MEDIUM

In case of sterile crosses, sucrose was used 0.2 g. per 100 ml and the percentage of the supplement was increased t0 50 mg /100 ml (Suyama *et al.* 1958).

Composition of Suyama's Crossing Medium Per Liter

1.	Distilled water	750ml
2.	Westergaard's stock solution	250ml
2.	Sucrose	2g
3.	Agar	20g

In the upper two cases, amino-acidwas added where necessary . For example in case of tryptophan mutants, 40 mg. of tryptophan per 1000ml was generally used.

The filter papers measurement 1.5″×2.5″ were folded thrice to make two furrows (W shaped) and introduced into the test tubes (6″) . Then 8-10 ml of the medium was poured in each crossing tube. The medium remained in furrows of the folded filter paper strip when the tubes were slanted.

3.7 STERILIZATION

All the media and essential instruments were sterilized in the autoclave 120^{0}C under 15 1b pressure for 20 minutes. The inoculation chamber, needle and the working space were sterilized with rectified spirit and the needle was burnt over the flame of a spirit lamp.

METHODS

4. METHODS

4.1 STOCK MAINTAINING

The stock of the cultures of the mutants,markers, wild types (EmA and Ema) were maintained in stock tubes containing 5 ml medium with necessary supplements (in case of mutants and markers). The wild type could grow in Vogel's minimal medium without any supplement. After incubation period, when cultures produce sufficient conidia were preserved in a refrigerator at $\pm 4^0$ C. The cultures were found to produce sufficient conidia within 4 to 5 days.

4.2 SUBCULTURING

Generally $2^1/_2{}''- 3''$ small tubes containing 2 ml of sterilized media was used for sub culturing. Vogel's supplemented medium (where supplement was necessary) were used for subculturing. Supplements were added at the time of preparation of media. Vogel's minimal medium was used for subculturing wild types and morphological mutants of *Neurospora crassa* and the medium was supplemented with 10 mg/100 ml media of necessary amino-acid for subculturing biochemical mutants and the markers.

Subculturing was done in a sterilized inoculation chamber. The inoculation chamber, the inoculation needle and the hands were first sterilized with rectified spirit. The inoculation needle was sterilized by heating over the flame of a spirit lamp kept in the chamber and then cooled into the new medium before each time of transfer the inoculum. Inoculum was transferred from old stock to new medium.

The culture tube then incubated at 25^0C for 4-5 days for the growth and formation of conidia.

4.3 SPONTANEOUS MUTATION IN *Neurospora Crassa*

Spontaneous mutation in *Neurospora crassa* is very rare and in frequent in nature (Stadler D *et. al,* 1993) It was checked if there was any spontaneous mutation in *Neurospora crassa.* Several steps were taken for finding out the spontaneous mutation in *Neurospora crassa.*

i) Preparation of solutions of conidial suspention of Ema

4-5 days old fresh culture of *N. crassa* was used to preparation of conidial suspension. 16 ml sterilized distilled water was taken in a large test tube. A small loop of conidia of Ema were taken and put into the tube containing sterilized distilled water. Then the tube was shaken well to make a homogenous suspension of conidia.

ii) Centrifugation

16 ml of the suspension was taken in two centrifuge tubes, 8ml in each and the two centrifuge tubes were placed in the centrifuge machine. So that two centrifuge tubes remain opposite to each other. Then the suspension was centrifuged for 10 minutes.

iii) Preparation of suspension of the treated conidia

After centrifugation the solution, above conidia was poured out (2-3 time) from the centrifuge tubes 10 ml of sterile distilled water was

added to each tube. Then the tubes were shaken well for mixing the water with conidia properly.

iv) Plating

1 drop of conidial suspension were taken in sterilized petriplates (100 plates) by sterilized pipette 10ml of molten SM of 47^{0}C was added to each petriplates and shaken carefully for proper distribution of conidia.

V) Incubation

The petriplates were incubated at 25^{0}C for 18-24 hours

Vi) Isolation

After growth of conidial colonies in SM petriplates, single conidial colonies were isolated in fresh and sterilized VM in 1000 tubes with isolating needle. The isolates were incubated at 25^{0}C for proper growth .

Vii) Observation

The culture were observed after 5 days if there was any morphological changes of mycelia conidia and its colour in comparison with Ema.

4.4 PREPARATION OF SOLUTION OF DIFFERENT CONCENTRATIONS OF LEAF EXTRACT OF *Adhatoda zeylanica* Nees.

At first of 220g. fresh leaves of *Adhatoda zeylanica* Nees. was measured with the help of balance. The leaves were washed with tap water 2-3 times and finally it was washed with distilled water . A

paste was made with the help of a grinder. The paste was filtered 2-3 times with the help of sterilized thick cotton cloth and finally it was filtered with the help of sterilized filter paper. The whole filtration was done in an inoculation chamber to avoid contamination.

Different extract of known strengths were prepared using the following leaf extract and sterilized distilled water.

10 ml leaf extract without distilled water is denoted as A_0. The next solution of 10 ml extract and 10 ml distilled water (A_1), 10 ml extract and 15 ml distilled water (A_2), 10 ml extract and 20 ml distilled water (A_3)

All concentration namely A_0, A_1, A_2, and A_3 were preserved separately in sterilized test tube in refrigerator. Some of extract were also sterilize in autoclave.

4.4.1 EFFECT OF NON-STERILIZED AND STERILIZED LEAF EXTRACT OF *Adhatoda zeylanica* Nees. ON THE GROWTH OF *Neurospora crassa.*

At first the petridishes were marked as o drop (conrol), 1 drop, 2 drops, 0.5 ml, 1 ml and 2ml with the help of glass marking pen. 1 drop, 2 drops, 0.5 ml, 1 ml and 2 ml of non-sterilized and sterilized A0, A_1, A_2 and A_3 concentration were taken in separate sterilized petridishes with the help of sterilized pipette. 10 ml of molten VM plating medium was added in each petridish and the petridishes were shaken gently for through mixing of the extract. As for control VM

plating medium was taken in each sterilized petidish. When the petridishes were cooled 4 days old Ema was inoculated at the centre of the petridishes inside the inoculation chamber to prevent contamination. Test for both control and the leaf extract concentration was done in duplicate.

The petridishes were kept in the incubator at 25^0C and observed after 18,24,30,36 and 42 hours.

4.4.2 ARTIFICIAL INDUCTION OF MUTATION IN *Neurospora crassa* WITH LEAF EXTRACT OF *Adahatoda zeylanica* Nees.

1. Obtaining of fresh culture of Ema

For obtaining of fresh culture Ema was subcultured 4 times after an interval of 4 days. In each case culture of 5 days old was used for treating conidia.

2. Sterilization

Inoculation chamber, needle, hands, centrifuge tubes and centrifuge machine were sterilized with rectified spirit. The centrifuge tubes were then washed with sterilized distilled water. Media, petridishes, tubes with pipette and distilled water were sterilized previously in the autoclave at 121^0C, 15 lb pressure for 20 minutes.

3. Centrifugation

10 ml of the solution of leaf extract of *Adhatoda zeylanica* Nees, of the concentration A_0 and A_1 were taken into 2 cnetrifuge tubes marked A_0 and A_1 with the help of glass marking pen. 1 loop of conidia of Ema was given to A_1 tube and waited for 10 minutes. After 10 minutes 1 loop of conida of Ema was given to A_0 tube. The tubes were shaken gently for homogenious solution. The tubes with suspension were then placed into centrifuge machine and centrifuged for 10 minutes.

4. Washing

After a fixed period of time of centrifugation the solution was poured out from the centrifuge tube. So that the conidia could remain at the bottom of the centrifuge tube. Then equal amount of sterilized distilled water was added in the centrifuge tubes and again the conidia were centrifuged for 3 to 5 minutes.

5. Preparation of suspension with treated conidia

10ml of sterilized distilled water was added to the treated conidia remaining at the bottom of the centrifuge tube. Then the tube was shaken well, for mixing the water with conidia properly.

6. Plating of treated conidia

The sterilized petridishes were marked as A_0, A_1, and 1 drop of the suspension was taken accordingly. 10 ml of molten SM medium was added to a petridish and were shaken gently to mix the suspension and medium.

7. Incubation

The paltes were kept inside the incubator at 25^0C for growth of conidia.

8. Isolation of single conidial colony

A number of well separated colonies were isolated by cutting agar block from the conidial colony with an arrow shaped isolating needle and were inoculated into small tubes containing VM media precaution were taken so that only the distinctly separated conidial colonies wee isolated.

9. Incubation

The cultures were incubated at a temperature of 25^0C.

10. Classification

The cultures were observed after 5 days and classified by comparing their characters with wild types Ema.

4.5 PREPARATION OF SOLUTION OF DIFFERENT CONCENTRATIONS OF LEAF EXTRACT OF *Centella asiatica* (Linn.) Urban.

At first 190g. of fresh leaves of *Centella asiatica* (Linn) was measured with the help of a balance. The leaves were washed with tap water 2-3 times and finally washed with distilled water. A paste was made with the help of a grinder. The paste was filtered 2-3 times with the help of sterilized thick cotton cloth and finally it was filtered with the help of sterilized filter paper. The filtration was done in the inoculation chamber to avoid contamination.

Different extract of known strengths were prepared using the following leaves and sterilized distilled water.

10 ml leaf extract without distilled water is denoted as (C_0), the next solution of 10 ml extract and 10 ml distilled water (C_1), 10 ml extract and 15 ml distilled water (C_2), 10 ml extract and 20 ml distilled water (C_3).

All extract namely C_0, C_1, C_2 and C_3 were preserved separately in sterilized test tube in refrigerator. Some of extract were also sterilized in autoclave.

4.5.1 EFFECT OF NON-STERILIZED AND STERILIZED LEAF EXTRACT OF *Centella asiatica* (Linn.)Urban ON THE GROWTH OF *Neurospora crassa.*

At first the petridishes were marked as o drop (control), 1 drop, 2 drops, 0.5ml, 1 ml and 2 ml with the help of glass marking pen. 1 drop,2 drop, 0.5 ml, 1 ml and 2 ml of non-sterilized and sterilized C_o, C_1, C_2 and C_3 concentration were taken in separate sterilized petridishes with the help of sterilized pipette. 10 ml of molten VM plating medium was added in each petridish and the petridishes were shaken gently for through mixing of the extract. As for control VM plating medium was taken in each sterilized petridish. When the petridish was cooled 4 days old Ema was inoculated at the centre of the petridishes inside the inoculation chamber to prevent contamination. Test for both control and the leaf extract concentration was done in duplicate .

The petridishes were kept in the incubator at 25^0C and observed after 18,24,30,36, and 42 hours.

4.5.2 ARTIFICIAL INDUCTION OF MUTATION WITH LEAF EXTRACT OF *Centella asiatica* (Linn.)Urban. IN *Neurospora crassa.*

1. Obtaining of fresh culture of Ema

For obtaining of fresh culture Ema was subcultured 4 times after an interval of 4 days. In each case culture of 5 days old was used for treating conidia.

2. Sterilization

Inoculation chamber, needle, hands, centrifuge tubes, centrifuge machine were sterilized with rectified spirit. The centrifuge tubes were then washed with sterilized distilled water. Media, petridishes, tubes with pipettes and distilled water sterilized previously in the autoclave at 121^0C under 15 1b pressure for 20 minutes.

3. Centrifugation

10 ml of the solution of leaf extract of *Centalla asiatica (*Linn.) Urban of the concentration C_0 and C_1 were taken into 2 cnetrifuge tubes marked C_0 and C_1 with the help of glass marking pen. 1 loop of conidia of Ema was given to C_1 tube and waited for 10 minutes. After 10 minutes 1 loop of conida of Ema was given to C_0 tube. The tubes were shaken gently for homogenious solution. The tubes with

suspension were then placed into centrifuge machine and centrifuged for 10 minutes.

4. Washing

After a fixed period of time of centrifugation, the solution was poured out from the centrifuge tube. So that the conidia could remain the bottom of the centrifuge tube. Then equal amount of sterilized distilled water was added in the centrifuge tubes and again the conidia were centrifuged for 3 to 5 minutes.

5. Preparation of suspension treated conidia

10 ml of sterilized distilled water was added to the treated conidia remaining at the bottom of the centrifuge tube and the tube was shaken well.

6. Plating of treated conidia

1–2 drop of these suspension was taken in each sterilized petridish and 10 ml of molten sorbose minimal medium (SM) was added in each petridish. The petridishes were marked as C_0 and C_1 previously. The temperature of the media did not exceed 47^0C. The suspension and media were mixed well by shaking the plate gently. Precautions were taken throughout the experiment to avoid contamination.

7. Isolation of single conidial colony

A number of well separated colonies were isolated by cutting agar block from the conidial colony with an arrow shaped islolating needle and were inoculated into small tubes containing VM media precaution

were taken so that only the distinctly separated conidial colonies were isolated.

8. Incubation

The cultures were incubated at a temperature of 25^0C

9. Classification

The culture were observed after 5 days and classified by comparing their characters with wild type Ema.

4.6 PREPARATION OF SOLUTION OF DIFFERENT CONCENTRATION OF FRUIT EXTRACT OF *Phyllanthus emblica* Linn.

At first 200g. fresh fruit of *Phyllanthus emblica* Linn. was measured with the help of a balance. The fruits were washed with tap water 2-3 times and finally it was washed with distilled water. A paste was made with the help of a grinder. The paste was filtered 2-3 times with the help of sterilized thick cotton cloth and finally it was filtered with the help of sterilized filter paper. The whole filtrations was done in a chamber to prevent contamination.

Different extract of known strengths were prepared using the following fruit extract and sterilized distilled water.

10 ml fruit extract without distilled water is denoted as P_0. The next solution of 10 ml extract and 10 ml distilled water (P_1), 10ml extract and 15 ml distilled water (P_2), 10 ml extract and 20 ml distilled water (P_3).

All concentration namely P_0, P_1, P_2 and P_3. Each concentration was taken in duplicate and one set was sterilized in the autoclaved at 121^0C under 15 1b pressure for 20 minutes and other set was kept at normal temperature of the laboratory.

4.6.1 EFFECT OF NON-STERILIZED AND STERILIZED FRUIT EXTRACT OF *Phyllanthus emblica* Linn. ON THE GROWTH OF *Neurospora crassa*.

Experiment was made to find out the effect of fruit extract of solution of *phyllathus enblica* Linn. on the growth of *Neurospora crassa*.

At first the petridishes were marked as 0 drop (control), 1 drop, 2 drops, 0.5 ml, 1 ml and 2 ml with the help of glass marking pen. 1 drop, 2 drops, 0.5 ml, 1 ml and 2 ml of non-steritzed and sterilized P_o, P_1, P_2, and P_3 concentration were taken in separate sterilized petridishes with help of sterilized pipette. 10 ml of molten VM plating medium was added in each petridishes and the petridishes were shaken gently for through mixing of the extract. As for controlVM plating medium was taken in each sterilized petridish. When the petridishes were cooled 4/5 days old Ema was inoculated at the center of the petridishes inside the inoculation chamber to prevent contamination. Test for both control and the fruit extract concentration was done in duplicate.

The petridishes were kept in the incubator at 25^0C and observed after 18,24,30,36, and 42 hours.

4.6.2 ARTIFICIAL INDUCTION OF MUTATION WITH FRUIT EXTRACT OF *Phyllanthus emblica* Linn.IN *Neurospora crassa.*

1.Obtaining of fresh culture of Ema

For obtaining of fresh culture Ema was subcultured 4 times after an interval of 4 days. In each case culture of 5 days old was used for treating conidia.

2. Sterilization

Inoculation chamber, needle, hands centrifuge tubes and centrifuge machine were sterilized with rectified spirit. The centrifuge tubes were then washed with sterilized distilled water, media, petridishes, tubes with pipettes and distilled water were sterilized previoulsy in the autoclave at 121^0C under 15 1b pressure for 20 minutes.

3. Centrifugation

10 ml of the solution of fruit extract of *phyllanthus emblica* Linn. of the concentration P_0 and P_2 were taken into 2 centrifuge tubes marked P_0 and P_2 with the help of glass marking pen. 1 loop of conidia of Ema was given to P_2 tube and waited for 10 minutes. After 10 minutes 1 loop of conidia of Ema was given to P_0 tube. The tubes were shaken gently for homogenous solution. The tubes with suspension were then placed into centifuge machine and centrifuged for 10 minutes.

4. Washing

After a fixed period of time of centrifugation, the solution was poured out from the centrifuge tube. So that the conidia could remain at the bottom of the centrifuge tube. Then equal amount of sterilized distilled water was added in the centrifuge tubes and again the conidia were centrifuged for 3 to 5 minutes.

5. Preparation of suspension with treated conidia

10 ml of sterilized distilled water was added to the treated conidia remaining at the bottom of the centrifuge tube. Then the tube was shaken well.

6. Plating of treated conidia

1 drop of these suspension was taken in each sterilized petidish and 10 ml of molten sorbose minimal medium(SM) was added in each petridish. The petridishes were marked as P_0 and P_2 previously. The temperature of the media did not exceed 47^0C. The suspension and media were mixed well by shaking the plate gently. Precautions were taken throughout the experiment to avoid contamination.

7. Incubation

The petridishes were kept inside the incubator at 25^0C for growth of conidia.

8. Isolation

When the growth was visible on the media, the distinctly separated conidial colonies were isolated. A part of the well separated colony

was cut with agar surface by isolating needle and was inoculated into culturing test tubes containing VM media.

9. Incubation

The cultures were incubated at a temperature of 25^0C

10. Classification

The cultures were observed after 5 days and classified by comparing their characters with wild types Ema.

4.7. SELECTION OF SOME MUTANTS

Mutants form different groups wee subcultured for three times and observation was taken whether there was any change in their characters. The mutants which retain their characters every time were finally selected for further studies such as linear growth, radial growth, mycelia growth, growth pattern, conidial germination crossing and linkage.

4.8 STUDY OF COMPARATIVE MYCELIAL GROWTH

For study of comparative mycelial growth, mutants and wild types of *Neurospora crassa* were cultured in liquid VM medium. 50 ml of medium was taken in each of small flasks and was autoclaved. The mutants and wild types were inoculated in the flask. The flasks were shaken continuously to prevent formation of conidia, Weight of empty filter papers were taken first. When the cultures were 72 hours old, flasks with cultures were heated in water for 2 minutes and were dried for two days in the oven. Precaution was taken so that the cultures did

not bunt. Then the weight of filter papers with culture were taken. The actual result was found by subtracting the weight of empty filter paper from the weight of filter paper containing dried mycelia.

4.9 STUDY OF LINEAR GROWTH

The cultures were inoculated in race tubes (Ryan *et al* 1943). The race tubes were made of long pyrex glass tubes. The horizontal portion was kept 40 cm with an internal diameter of about 14 m.m. One side of the race tube was bent up at an angle of 45^0 and the opposite side at 90^0 angles. The openings were fire polished and flared slightly.

These tubes were held up right on a flat surface with clips. The horizontal portions of the growth tubes were marked in millimeters. Flat portions of the tubes were half filled with the solid vogels minimal medium containing 4% of agar. Inoculaton was performed at one end of the medium inside the inoculation chamber.

4.10 CROSSING

Neurospors crassa strains are heterothallic with two mating types 'A' and 'a' According to Ahmad, M. (1953) heterothallics are defined as the types in which the initiation and/ or consummation of sexual reproduction is deferred in uninucleate single spore cultures till they come across the complementary strain.

Sexual mating bodies called perithecia are produced only when two mating types are together on a suitable media (Shear and Dodge, 1927; Westergaard and Mitchell 1947; Anonymous 1966) Westergaard's

crossing medium was usually used for crossing. In some case when the crosses were sterile i.e. did not form Perithecia then Suyama's crossing medium was used.

Crossing was made in a big tube (6″) containing 6-8 ml of westrgaard's crossing medium. One strain was put on one through of the filter paper pleat and the other was put on the other through of the pleat. The crosses were then incubated at 25^0C.

Where the mutants were fertile, perithecia formed in a week, most times the appearance of perithecia was seen in five days very rarely after 10 days or 12 days.

Perithecia generally liberated spores within 15 days. Where there was the delay of the formation of perithecia hardly their was the shedding of the spores.

If the crosses were sterile, the quantity of sugar in the medium was reduced to 0.25% and the amino-acid supplement was increased to 50 mg per 100 ml of the medium in accordance with the findings of Suyama *et al* (1958).

The morphological mutants were crossed with wild types 'Ema' and 'EmA' for checking the mating types and they are crossed with biochemical markers of seven linkage groups for the study of linkage group.

Improvement Of Fertility Of The Mutants

i) The mutants were subcultured four times after 4 days interval to improve their fertility .

ii) Rapid subcultured mutants were crossed in westergaard's medium containing 50 mg percent amino-acid with the wild types or the marker.

iii) The wild types or the markers as the case might be, were subcultured four times before crosses were made.

iv) For good conidia formation, the amount of tryptophan was used 50 mg / 100 ml of the media.

v) The crosses were incubated at 25^0C bath.

4.11 STUDY OF SEGREGATION AND LINKAGE.

When the cross between mutant and wild had formed perithecia and the spores had shaded then the segregation was studied. Spores were spread and individual spores were grown on culture media and then incubated. Single spores were inoculated separately in small test tubes and they grew up. When the cultures attained maturity, some isolates showed similarity with the characters of the mutants and others with wild type. The ratio between mutants isolates and wild types isolates was calculated. In most cases, it was found to be about 1:1.

Then a number of isolates from two grops (mutant and wild types isolates) were crossed with 'Ema' and 'EmA'. The isolates that formed perithecia with 'Ema' was the strain 'A' and the strain which

formed perithecia with 'EmA' was the strain 'a' . In many cases the ratio between strain 'a' and 'A' was about 1:1.

For the study of segregation the following procedure was followed.

i. Spore spreading
ii. Isolation of single spores
iii. Incubation of the isolates
iv. Analysis of the isolates
v. Checking the mating types of the isolates checking.

i) Spore spreading

When plenty of spores had shed the cross was ready for spreading. A spreading needle made of glass rod bent twice at right angles was boiled for 20 minutes. 2-3 drops of sterilized distilled water was put on SM plates with sterile pipette. Some places on the crossing tube with spores were marked with the help of glass marking pencile under microscope. A wire loop needle was burnt red heat and cooled in sterilized distilled water plate. With the help of the cooled loop needle, one loop of ascospores was taken from the marked place of desired cross and was transfered to the distilled water drops on SM plate. The sterilized spreading needle was then cooled in sterilized distilled water plate and the water with spores was spreader uniformly with the help of the cooled spreading needle. The spreading needle was again boiled for sterilization. The name of the cross was written on the upper lid of the petridish. The prepared plate was then given a heat shock at 58^0C for 50 minutes and then incubated at 25^0C 12-16 hours as necessary.

ii) Isolation of single spores

Usually 12-16 hours few spores were growing and few were germinating. The spores that formed well-separated colonies and did not come in any contact with others were isolated with isolating needle and inoculated separately in small tubes containing culture media (VM or supplemented VM as needed). A good number of spores were isolated randomly.

Precautions were taken so that only the distinctly separated spores were isolated.

iii) Incubation

The tubes with inoculum were then incubated at 25^0C for growth of mycelia and conidia.

iv) Analysis of the isolates

After 4 to 6 days the isolates were then analyzed whether they showed similarity with the characteristics of mutants or wild types. The ratio between the mutant isolates and wild like isolates was calculated.

v) Checking the mating types of the isolates

The selected isolated were chosen and their mating types were determined by crossing them with the wild types of both Ema and EmA. If perithecia formed with 'Ema' the mating type of the extract would be A and if perithecia formed with 'EmA' the mating type of the extract would be "a". If perithecia were not formed the cross become sterile.

EXPERIMENT AND RESULTS

5. EXPERIMENT AND RESULTS

5.1 TO FIND OUT IF THERE WAS ANY SPONTANEOUS MUTATION IN *Neurospors crassa.*

Treating of Ema : Conidia from Ema of 4 days old were treated with sterilized distilled water for 10 minutes. SM was used for plating and VM was used for single conidial isolation.

Observation : After 14 hours about 1000 single conidial colonies were isolated. The cultures were incubated at 25^0C for growth of mycelia and conidia. After 5 days the isolates were observed if there was any abnormalities in their characters by comparing with that of Ema.

Conclusion : Among those, 1000 isolates observed there was no change in morphological features of *Neurospora crassa*. So it seems that there was no spontaneous mutation in *Neurospora crassa* in our laboratory stock which we used.

5.2. PREPARATION OF DIFFERENT CONCENTRATIONS OF LEAF EXTRACT OF *Adhatoda zeylanica* NEES.

Different concentrations of leaf extract of *Adhatoda zeylanic* Nees. were prepared with distilled water.

Table-1

Different concentrations of leaf extract of *Adhatoda zeylanica* Nees.

Serial number	Amount of leaf extract used	Amount of sterilized distilled water.	Name of the concentrations
1	10 ml	-	A_o
2	10 ml	10 ml	A_1
3	10 ml	15 ml	A_2
4	10 ml	20 ml	A_3

5.2.1 EFFECT OF NON-STERILIZED AND STERILIZED LEAF EXTRACT OF *Adhatoda zeylanica* Nees. ON THE GROWTH OF *Neurospora crassa.*

The petridishes were marked as o drop (control), 1 drop, 2 drops, 0.5 ml, 1 ml and 2 ml of non-sterilized and sterilized A_0, A_1, A_2, and A3 concentrations were taken in each sterilized petridish. 10 ml of molten VM plating media was added to each petridish. When the petridish was cooled, Ema was inoculated at the centre of the petridish. The petridisehes were kept in the incubator at 25^0C and observed after 18, 24, 30, 36 and 42 hours and the radial growth of the colony was measured in centimeter. The results were given in Table-2, 3, 4, 5, 6, 7, 8, 9.

Table-2

Effect of non-sterilized A_0 concentration of leaf extract of *Adhatoda zeylanica* Nees. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of non-sterilized A_O concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	2.8 2.9	2.85	1.5 1.6	1.55	0.6 0.7	0.65	0 0	0	0 0	0	0 0	0
24	3.0 3.1	3.05	2.0 2.2	2.1	0.9 0.9	0.9	◉ ◉	◉	◉ ◉	◉	0 0	0
30	3.4 3.6	3.5	2.8 2.9	2.85	1.3 1.4	1.35	0.4 0.4	0.4	0.3 0.2	0.25	◉ ◉	◉
36	4.2 4.5	4.35	3.0 3.5	3.25	1.9 1.8	1.85	0.8 0.9	0.85	0.7 0.6	0.65	0.3 0.2	0.25
42	5.3 5.5	5.4	4.0 4.0	4.0	2.8 2.6	2.7	1.4 1.5	1.45	1.2 0.9	1.05	0.5 0.5	0.5

Note : O Indicates no growth
◉ Indicates some growth

Table-3

Effect of sterilized A_0 concentration of leaf extract of *Adhatoda zeylanica* Nees. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of sterilized A_O concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	2.8 2.9	2.85	1.8 1.9	1.85	0.9 0.9	0.9	◉ ◉	◉	◉ ◉	◉	0 0	0
24	3.0 3.1	3.05	2.4 2.3	2.35	1.3 1.6	1.45	0.8 0.9	0.85	0.4 0.5	0.45	◉ ◉	◉
30	3.4 3.6	3.5	3.0 3.0	3.0	2.5 2.5	2.5	1.2 1.3	1.25	1.3 1.5	1.4	0.6 0.7	0.65
36	4.2 4.5	4.35	3.6 3.6	3.6	2.9 2.8	2.85	2.7 2.6	2.65	1.6 1.56	1.55	1.3 1.2	1.25
42	5.3 5.5	5.4	4.2 4.2	4.2	3.0 3.2	3.1	2.9 3.0	2.95	1.8 1.9	1.85	1.6 1.6	1.6

Note : O Indicates no growth
◉ Indicates some growth

Table-4

Effect of non-sterilized A_1 concentration of leaf extract of *Adhatoda zeylanica* Nees. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of non-sterilized A_1 concentration											
	o drop (control)		1drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	2.8 2.9	2.85	1.6 1.7	1.65	1.0 1.0	1.0	0.6 0.6	0.6	0.3 0.4	0.35	◉ ◉	◉
24	3.0 3.1	3.05	0.9 2.1	1.5	1.2 1.4	1.3	1.0 1.2	1.1	0.7 0.6	0.65	0.3 0.3	0.3
30	3.4 3.6	3.5	2.3 2.4	2.35	1.8 1.7	1.75	1.4 1.6	1.5	1.0 1.2	1.1	0.5 0.6	0.55
36	4.2 4.5	4.35	2.7 2.8	2.75	2.0 2.0	2.0	2.0 2.2	2.1	1.4 1.3	1.35	0.8 0.9	0.85
42	5.3 5.5	5.4	3.6 3.7	3.65	2.9 2.7	2.8	2.5 2.3	2.4	1.8 1.7	1.75	1.2 1.3	1.25

Note: ◉ Indicates some growth

Table-5

Effect of sterilized A_1 coneentration of leaf extract of *Adhatoda zeylanica* Nees. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of sterilized A_1 concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	2.8 2.9	2.85	1.9 1.8	1.85	1.5 1.4	1.45	1.2 0.9	1.05	0.8 0.9	0.85	0.5 0.4	0.45
24	3.0 3.1	3.05	2.2 2.2	2.2	1.7 1.6	1.65	1.4 1.4	1.4	0.9 1.1	1.0	0.7 0.5	0.6
30	3.4 3.6	3.5	2.5 2.4	2.45	2.0 2.1	2.05	1.8 2.0	1.9	1.5 1.4	1.45	0.8 0.9	0.85
36	4.2 4.5	4.35	3.0 3.0	3.0	2.5 2.2	2.35	2.0 2.2	2.1	1.9 1.8	1.85	1.2 1.1	1.15
42	5.3 5.5	5.4	4.5 4.6	4.55	3.0 3.0	3.0	2.7 2.6	2.65	2.0 2.0	2.0	1.5 1.6	1.55

Table-6

Effcet of non-sterilized A_2 concentration of leaf extract of *Adhatoda zeylanica* Nees. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of non-sterilized A_2 concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	2.8 2.9	2.85	2.3 2.2	2.25	1.8 1.9	1.85	1.4 1.5	1.45	1.0 1.2	1.1	0.6 0.5	0.55
24	3.0 3.1	3.05	2.5 2.5	2.5	2.2 2.1	2.15	1.8 1.7	1.75	1.5 1.3	1.4	0.8 0.8	0.8
30	3.4 3.6	3.5	2.8 2.7	2.75	2.4 2.4	2.4	2.0 2.2	2.1	1.8 1.7	1.75	1.2 1.1	1.15
36	4.2 4.5	4.35	3.0 2.9	2.95	2.7 2.8	2.75	2.6 2.7	2.65	2.2 2.1	2.15	1.6 1.4	1.5
42	5.3 5.5	5.4	4.0 3.9	3.95	3.6 3.7	3.65	3.0 3.2	3.1	2.7 2.7	2.7	2.0 2.1	2.05

Table-7

Effect of sterilized A_2 eoncentration of leaf extract of *Adhatoda zeylanica* Nees. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of sterilized A_2 concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	2.8 2.9	2.85	2.4 2.3	2.35	1.9 1.9	1.9	1.6 1.7	1.65	1.5 1.6	1.55	0.9 0.8	0.85
24	3.0 3.1	3.05	2.8 2.7	2.75	2.0 2.2	2.1	1.9 2.0	1.95	1.8 1.7	1.75	1.0 1.1	1.05
30	3.4 3.6	3.5	3.1 3.0	3.05	2.6 2.7	2.65	2.2 2.2	2.2	2.1 2.1	2.1	1.6 1.5	1.55
36	4.2 4.5	4.35	3.8 3.9	3.85	3.0 3.2	3.1	2.7 2.6	2.65	2.3 2.4	2.35	1.8 1.9	1.85
42	5.3 5.5	5.4	4.2 4.4	4.3	3.9 3.9	3.9	3.7 3.7	3.7	3.0 3.1	3.05	2.6 2.7	2.65

Table-8

Effeet of non-sterilized A_3 concentration of leaf extract of *Adhatoda zeylanica* Nees. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of non-sterilized A_3 concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	2.8 2.9	2.85	2.5 2.4	2.45	2.2 2.0	2.1	1.8 1.9	1.85	1.5 1.4	1.45	0.5 0.6	0.55
24	3.0 3.1	3.05	2.8 2.9	2.85	2.6 2.4	2.5	2.0 2.1	2.05	1.8 1.9	1.85	0.8 0.9	0.85
30	3.4 3.6	3.5	3.2 3.2	3.2	2.8 2.9	2.85	2.3 2.4	2.35	1.9 2.0	1.95	1.5 1.3	1.4
36	4.2 4.5	4.35	3.9 4.0	3.55	3.2 3.2	3.2	2.9 2.9	2.9	2.4 2.5	2.45	1.8 1.8	1.8
42	5.3 5.5	5.4	4.9 4.7	4.8	4.7 4.8	4.75	3.6 3.7	3.65	3.4 3.4	3.4	2.8 2.7	2.75

Table-9

Effect of sterilized A_3 concentration of leaf extract of *Adhatoda zeylanica* Nees. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of sterilized A_3 concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	2.8 2.9	2.85	2.8 2.7	2.75	2.3 2.4	2.35	1.9 2.1	2.0	1.6 1.7	1.65	0.8 0.7	0.75
24	3.0 3.1	3.05	3.0 2.9	2.95	2.6 2.6	2.6	2.3 2.4	2.35	1.9 2.0	1.95	1.2 1.3	1.25
30	3.4 3.6	3.5	3.4 3.4	3.4	2.9 2.8	2.85	2.8 2.7	2.75	2.2 2.3	2.25	1.8 1.9	1.85
36	4.2 4.5	4.35	4.5 4.4	4.45	3.6 3.6	3.6	3.7 3.6	3.65	3.0 3.2	3.1	2.0 2.2	2.1
42	5.3 5.5	5.4	5.0 5.0	5.0	4.8 4.9	4.85	4.2 4.3	4.25	3.8 3.9	3.85	3.5 3.4	3.45

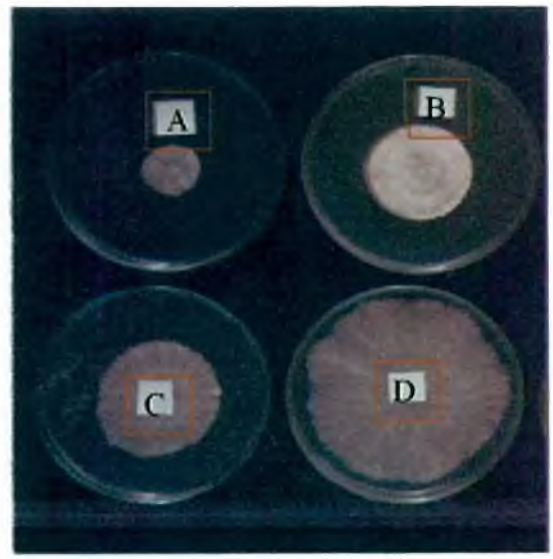


Fig :1.Effect of different doses of non-sterilized A_0 concentration of *Adhatoda zeylanica* Nees. on the growth of *N. crassa*. **A.**1ml, **B**. 0.5 ml, **C.** 2 drops and **D.** 1 drop.

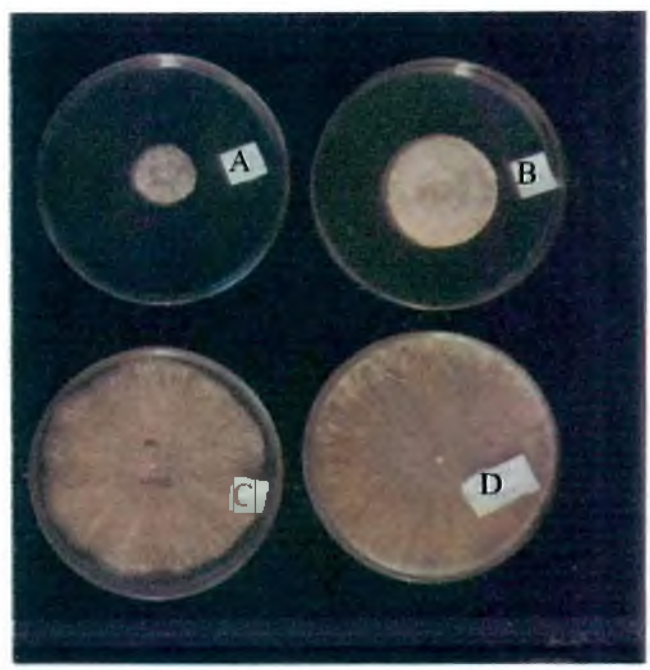


Fig: 2.Effect of different doses of non-sterilized A_1 concentration of *Adhatoda zeylanica* Nees. on the growth *Neurospora crassa* **A**. o.5ml, **B.** 2 drops, **C**. 1 drop and **D**. 0 drop (control).

Conclusion : From table 2,3,4,5,6,7,8, and 9 it was found that, the concentrations of leaf extract of *Adhatoda zcylainca* Nees. control the radial growth of wild type (Ema) *Neurospora crass.* The result also showed that non-sterilized concentration was more effective than sterilized one. Non-sterilized A_0 concentration was the most effective and sterilized A_3 concentration was the least effective.

So, non-sterilized A_0 and A_1 concentration of leaf extract of *Adhatoda zeylanica* Nees. were selected for treating wild type (Ema) conidia.

5.2.2 STUDY OF MUTAGENIC EFFECT OF LEAF EXTREACT OF *Adhatoda zeylanica* Nees. IN *Neurospora crassa*

Leaf extract of different concentrations were used to test the mutagenic effect on *Neurospora crassa.* Certain concentration were effective. These concentrations had appropriate mutagenic effect on *Neurospora crassa.*

5.2.3 INDUCTION OF MUTATION WITH LEAF EXTRACT (*Adhatoda zeylanica* NEES.) SOLUTION OF THE CONCENTRARTION A_0 AND A_1

Conidia of Ema of 5 days old culture was treated with leaf extract (*Adhatoda zeylanica* Nees.) concentration of A_0 and A_1 for 10 minutes. SM was used for plating and VM in small tubes was used as culturing media. Plates with treated conidia were incubated at 25^0C. Single conidial colonies were isolated after 48 hours of incubation.

Then the cultures were incubated at 25^0C and observed after 5 days. The cultures that shows abnormality with Ema of same age were denoted as mutants. The mutants were different from Ema in colour of conidia mode of conidia mycelial structure etc.

5.2.4 CLASSIFICATION AND NOMENCLATURE OF OBTAINED MUTANTS INDUCED WITH LEAF EXTRACT OF *Adhatoda zeylanica* NEES.

Group- A: ropy

Mycelia form rope like structure conidia pink, growth less than wild type.

The following 11 mutants fall under this group.

A_0 305, A_0 307 A_0 308, A_0 310, A_1 420, A_1 424, A_1 440, A_1 445, A_1 510, A_1 512, A_1 525.

Group- B: cauliflower

Restricted growth mycelia and conidia form a cauliflower like structure.

The following 6 mutants fall under this group.

A_0 302, A_0 321 A_0 340 , A_1 411, A_1 415, A_1 501,

Group- C: crescent

Conidial and mycelial growth form a nice crescent at the tip of the growth mycelia colourless and steady in the tube, conidia light pink.

The following 16 mutants fall under this group.

A_0 330, A_0 345 A_0 346 , A_0 350, A_0 400, A_0 403, A_0 409, A_0 432, A_0-449, A_0 450, A_1 508, A_1 515, A_1 522, $A_1$605, A_1 609, A_1 620,

Group- D: checked

Very check growth mycelia more or less colourless hypae very small, growth smaller than the wild type conidia not purely orange in colouration.

The following 5 mutants fall under this group.

A_0 372, A_0 380 $A_1$444, $A_1$460, $A_1$465.

Group- E: vigorous

Profuse growth of mycelia and conidia, conidia are pinkish orange, mycelial and conidial growth is heavier than wild type.

The following 3 mutants fall under this group.

A_0 315, A_0 368 $A_1$550.

Table-10

Classification of mutants induced with leaf extract of *Adhatoda zeylanica* Nees.

Name of the group	Characteristics of the mutants	Concentration of the leaf extract of *Adhatoda zeylainca* Nees	Number of mutants	Name of the mutants
A	Mycelia form rope like structure, conidia pink	A_0 A_1	4 7	ropy
B	Growth forming a nice cauliflower like structure	A_0 A_1	3 3	cauliflower
C	Conidia growth form a nice crescent at the tip of the growth.	A_0 A_1	10 6	crescent
D	Growth less than wild	A_0 A_1	2 3	checked
E	Profuse conidial growth touches the plug	A_0 A_1	2 1	vigorous

5.3 PREPARATION OF DIFFERENT CONCENTRATIONS OF LEAF EXTRACT OF *Centella asiatica* (Linn.) Urban.

Different concentration of leaf extract of *Centella asiatic*(Linn.) urban. were prepared with distilled water.

Table 11

Different concentrations of leaf extract of *Centella asiatica* (Linn) Urban.

Serial Number	Amount of leaf extract used	Amount of sterilized distilled water	Name of the concentrations
1	10 ml	-	C_0
2	10 ml	10 ml	C_1
3	10 ml	15 ml	C_2
4	10 ml	20 ml	C_3

5.3.1 EFFECT OF NON-STERILIZED AND STERILIZED LEAF EXTRACT OF *Centella asiatica* (Linn.) Urban. ON THE GROWTH OF *Neurospora Crassa.*

o drop ,1 drop,2 drops 0.5 ml, 1 ml and 2 ml of non-sterilized and sterilized C_0, C_1, C_2,and C_3 concentrations were taken in each sterilized petridish. 10 ml of molten VM plating media was added to each petridish . When the petridish was cooled conidia from Ema was inoculated at the centre of the petridishes. The petridishes were kept in the incubator at 25^0C and observed after 18, 24, 30, 36, and 42 hours and the radial growth of the colony was measured in centimeter.The results were given in Table-12, 13, 14, 15, 16, 17, 18, 19.

Table-12

Effect of non-sterilized C_0 concentration of leaf extract of *Centella asiatica* (Linn.)Urban. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of non-sterilized Co concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	1.8 1.9	1.85	0.8 0.8	0.8	0.6 0.5	0.55	◉ ◉	◉	0 0	0	0 0	0
24	2.2 2.5	2.35	1.4 1.5	1.45	0.8 0.7	0.75	0.7 0.7	0.7	0 0	0	0 0	0
30	2.7 2.9	2.8	1.6 1.7	1.65	1.3 1.2	1.25	0.9 0.8	0.85	0.3. 0.5	0.4	◉ ◉	◉
36	3.5 3.8	3.65	1.0 1.9	1.8	1.5 1.7	1.6	1.4 1.3	1.35	0.6 0.7	0.65	0.5 0.4	0.45
42	4.2 4.4	4.3	2.0 2.3	2.15	2.0 2.2	2.1	1.8 1.9	1.85	0.9 0.8	0.85	0.7 0.8	0.75

Note : 0 indicates no growth
◉ indicates some growth

Table-13

Effect of sterilized C_0 concentration of leaf extract of *Centella asiatica* (Linn.)Urban. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of sterilized C_0 concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	1.8 1.9	1.85	0.9 0.8	0.85	0.7 0.8	0.75	0.7 0.6	0.65	0 0	0	0 0	0
24	2.2 2.5	2.35	1.7 1.8	1.75	1.0 1.2	1.1	1.0 0.9	0.95	◉ ◉	◉	◉ ◉	◉
30	2.7 2.9	2.8	1.9 1.9	1.9	1.5 1.4	1.45	1.3 1.2	1.25	0.6 0.6	0.6	0.5 0.4	0.45
36	3.5 3.8	3.65	2.0 2.2	2.1	1.8 1.8	1.8	1.5 1.3	1.4	0.9 0.8	0.85	0.7 0.7	0.7
42	4.2 4.4	4.3	3.5 3.4	3.45	2.3 2.5	2.4	1.9	1.85	1.0 1.2	1.1	0.9 0.9	0.9

Note : 0 indicates no growth
◉ indicates some growth

Table-14

Effect of non-sterilized C_1 concentration of leaf extract of *Centella asiatica* (Linn.)Urban. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of non-sterilized C_1 concentration											
	o drop (control)		1drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	1.8 1.9	1.85	1.0 1.1	1.05	0.6 0.7	0.65	0.4 0.4	0.4	0 0	0	0 0	0
24	2.2 2.5	2.35	1.5 1.6	1.6	1.3 1.2	1.25	0.9 1.0	0.95	0.3 0.3	0.3	◉ ◉	◉
30	2.7 2.9	2.8	1.9 1.9	1.9	1.6 1.6	1.6	1.2 1.3	1.25	0.8 0.7	0.75	0.3 0.3	0.3
36	3.5 3.8	3.65	2.3 2.5	2.4	1.9 1.9	1.9	1.8 1.9	1.85	1.3 1.4	1.35	0.8 0.9	0.85
42	4.2 4.4	4.3	3.9 3.7	3.8	2.8 2.9	2.85	2.3 2.3	2.3	2.0 1.9	1.95	1.0 1.1	1.05

Note : 0 indicates no growth
◉ indicates some growth

Table-15

Effect of sterilized C_1 concentration of leaf extract of *Centella asiatica* (Linn.) Urban on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of sterilized C_1 concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	1.8 1.9	1.85	1.1 1.2	1.15	0.8 0.9	0.85	0.6 0.6	0.6	0.3 0.4	0.35	0 0	0
24	2.2 2.5	2.35	1.6 1.7	1.65	1.5 1.6	1.55	1.0 1.2	1.1	0.5 0.6	0.55	0.4 0.4	0.4
30	2.7 2.9	2.8	2.0 1.9	1.95	1.8 1.9	1.85	1.5 1.4	1.45	1.2 1.1	1.15	0.6 0.6	0.16
36	3.5 3.8	3.65	2.6 2.6	2.6	2.0 2.1	2.05	1.9 1.10	1.5	1.5 1.6	1.55	1.3 1.3	1.1
42	4.2 4.4	4.3	4.0 4.2	4.1	3.5 3.6	3.55	2.2 2.1	2.15	2.2 2.3	2.25	1.7 1.8	1.75

Note : 0 indicates no growth

Table-16

Effect of non-sterilized C_2 concentration of leaf extract of *Centella asiatica* (Linn.) Urban on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of non-sterilized C_2 concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	1.8 1.9	1.85	1.3 1.3	1.3	1.2 0.9	1.05	0.8 0.9	0.85	0.5 0.4	0.45	◉ ◉	◉
24	2.2 2.5	2.35	1.8 1.7	1.75	1.5 1.4	1.45	1.0 1.2	1.1	0.8 0.7	0.75	0.75	0.5 0.5
30	2.7 2.9	2.8	2.0 2.1	2.05	1.8 1.9	1.85	1.4 1.4	1.4	1.2 1.1	1.15	0.7 0.6	0.65
36	3.5 3.8	3.65	2.8 2.9	2.85	2.0 2.0	2.0	1.9 1.9	1.9	1.5 1.6	1.55	1.2 1.3	1.25
42	4.2 4.4	4.3	3.8 3.9	3.85	3.0 2.9	2.95	2.5 2.6	2.55	2.1 2.2	2.15	1.8 1.9	1.85

Note : ◉ indicates some growth

Table-17

Effect of sterilized C_2 concentration of leaf extract of *Centella asiatica* (Linn.) Urban. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of sterilized C_2 concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	1.8 1.9	1.85	1.5 1.5	1.5	1.2 1.3	1.25	0.9 1.0	0.95	0.7 0.8	0.75	0.3 0.4	0.35
24	2.2 2.5	2.35	1.9 1.8	1.85	1.6 1.7	1.65	1.2 1.3	1.25	0.9 1.1	1.0	0.8 0.7	0.75
30	2.7 2.9	2.8	2.2 2.3	2.25	1.9 1.9	1.9	1.5 1.5	1.5	1.3 1.3	1.3	0.9 1.2	1.05
36	3.5 3.8	3.65	3.0 2.9	2.95	2.2 2.2	2.2	2.0 2.2	2.1	1.7 1.7	1.7	1.6 1.7	1.65
42	4.2 4.4	4.3	3.9 4.0	3.95	3.5 3.0	3.25	2.8 2.7	2.75	2.4 2.3	2.35	2.0 2.1	2.05

Table-18

Effect of non-sterilized C_3 concentration of leaf extract of *Centella asiatica* (Linn.)Urban. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of non-sterilized C_3 concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	1.8 1.9	1.85	1.6 1.5	1.55	1.5 1.4	1.45	0.8 0.9	0.85	0.6 0.6	0.6	0 0	0
24	2.2 2.5	2.35	`2.0 1.9	1.95	1.8 1.7	1.75	1.3 1.5	1.4	0.9 1.1	1.0	0.7 0.6	0.65
30	2.7 2.9	2.8	2.5 2.4	2.45	2.0 2.1	2.05	1.8 1.9	1.85	1.5 1.6	1.55	0.9 0.9	0.9
36	3.5 3.8	3.65	3.2 3.0	3.1	2.5 2.6	2.55	2.1 2.2	2.15	1.9 1.9	1.9	1.6 1.4	1.5
42	4.2 4.4	4.3	3.9 4.0	3.95	3.8 3.9	3.85	2.8 2.8	2.8	2.4 2.6	2.5	2.0 2.0	2.0

Note: 0 indicates no growth

Table-19

Effect of sterilized C_3 concentration of leaf extract of *Centella asiatica* (Linn.)Urban. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of sterilized C_3 concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	1.8 1.9	1.85	1.7 1.7	1.7	1.6 1.7	1.65	0.9 0.9	0.9	0.9 0.7	0.8	0.3 0.3	0.3
24	2.2 2.5	2.35	2.0 2.0	2.0	1.9 1.9	1.9	1.5 1.6	1.55	1.3 1.4	1.35	0.9 0.9	0.9
30	2.7 2.9	2.8	2.8 2.7	2.75	2.0 2.2	2.1	1.9 1.7	1.8	1.7 1.8	1.75	1.3 1.2	1.25
36	3.5 3.8	3.65	3.5 3.6	3.55	2.8 2.10	2.9	2.0 2.0	2.0	2.0 2.2	2.1	1.6 1.7	1.65
42	4.2 4.4	4.3	4.0 .4.1	4.05	3.9 3.9	3.9	3.0 3.1	3.05	2.5 2.6	2.55	2.4 2.4	2.4

Conclusion : From table 12,13,14,15,16,17,18 and 19 it was found that, the concentrtion of leaf extract of *Centella asiatica* (Linn.) Urban . control the radial growth of wild type (Ema) *Neurospora crassa.* The result also showed that sterilized concentration was less effective than non-sterilize one. Non-sterilized C_0 concentration was the most effect and steilized C_3 concentration was the least effective .

So, non-sterilized C_o and C_1 concentration were chosen for treating the conidia.

5.3.2 STUDY OF MUTAGENIC EFFECT OF LEAF EXTREACT OF *Centella asiatica* (Linn.) Urban. IN *Neurospora crassa*

Leaf extract of different concentrations were used to test the mutagenic effect on *Neurospora crassa.* Certain concentration were effective, These concentrationshad appropriate mutagenic effect on *Neurospora crassa.*

5.3.3 INDUCTIN OF MUTANTION WITH LEAF EXTRACT of *Centella asiatica* (Linn.) Urban. SOLUTION OF THE CONCENT-RATION C_0 ANDC_1

Conidia of Ema of 5 days old culture was treated with leaf extract (*Centella asiatica* Linn. Urban) concentration of C_0 and C_1 for 10 minutes. SM was used for plating and VM in small tubes was used as culturing media. Plates with treated conidia were incubated at 25^0C. Single conidial colonies were isolated after 48 hours of incubation.

Then the cultures were incubated at 25^0C and observed after 5 days . The cultures that shows abnormality with Ema of same age were denoted as mutants. The mutants were different from Ema in colour of conidia mode of conidia mycelial structure etc.

5.3.4 CLASSIFICATION AND NOMENCLATURE OF OBTAINED MUTANTS INDUCED WITH LEAF EXTRACT OF *Centella asiatica* (Linn.) Urban.

Group- 1: plug

Densed conidial growth inside the tube pink conidia formed outside the tube conidia around the plug.

The following 8 mutants fall into this group.

$C_0$20, $C_0$23, $C_0$32, C_1 112, C_1 125, C_1 136, C_1 201, C_1 215

Group- II : fluffy band

Mycelia colourless conidia very few or absent cottony mycelia formed a nice band of the tip of the growth.

The following 15 mutants fall into this group

$C_0$10, $C_0$12, $C_0$21, C_0 35, $C_0$42, $C_0$60, $C_0$64, $C_1$108, C_1 135, C_1 138, C_1 149, C_1 219, C_1 230, C_1 245, C_1 248.

Group- III : mat

Growth lie on the media like a mat. The growth can hardly be separated from the media, growth moistly .

The following 4 mutants fall into this group

C_o5, C_0 65, C_0 70, $C_1$150.

Group – IV Checked

Very checked growth, mycelia more or less colourless. Hyphae small, growth smaller than the wild, conidia not purely orange in colour.

The following 10 mutants fall into this group

$C_0$16, C_0 72, C_0 85, C_o163, C_o189, C_o 190, $C_1$194, C_1 258, C_1 267, C_1 271.

Table-20

Classification of mutants induced with leaf extract of *Centella asiatica* (Linn.) Urban.

Name of the group	Characteristics of the mutants	Concentration of the leaf extract of *Centella asiatica* (Linn.) Urban	Number of mutants	Name of the mutants
I	Conidia fromed outside the tube and over the plug	C_0 C_1	3 5	plug
II	Colourless mycelia form a band at the tip	C_0 C_1	7 8	fluffy band
III	Growth remain on the surface of media	C_0 C_1	3 1	mat
IV	Growth less than wild	C_0 C_1	6 4	checked

5.4 PREPARATION OF DIFFERENT CONCENTRATIONS OFFRUITEXTRACT OF *Phyllanthus emblica* Linn.

Different concentrations of Fruit extract of *Phyllanthus emblica* Linn. were prepared with distilled water.

Tabel-21

Different concentrations of Fruit extract of *Phyllanthus emblica* Linn.

Serial number	Amount of leaf extract used	Amount of sterilized distilled water	Name of the concentrations
1	10 ml	-	P_0
2	10 ml	10 ml	P_1
3	10 ml	15 ml	P_2
4	10 ml	20 ml	P_3

5.4.1 EFFECT OF NON-STERILIZED AND STERILIZED FRUIT EXTRACT OF *Phyllanthus emblica* Linn. ON THE GROWTH OF *Neurospora crassa*

For testing the effect different concentration (sterilized and non-sterilized) were taken separately on the sterilized petridishes at the rate of o drop (control), 1 drop, 2 drops, 0.5 ml, 1 ml and 2 ml. 10 ml of molten VM was added in each petridish. When media become solid Ema was inoculated at the centre of the media. The petridishes were kept in the incubator at 25^{0}C and observed after 18, 24, 30, 36 and 42 hours and the radial growth of the colony were measured in centimeter. The results were given in Table - 22, 23, 24, 25, 26, 27, 28, 29.

Table-22

Effect of non-sterilized P_0 concentration of fruit extract of *Phyllanthus emblica* Linn. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of non-sterilized P_0 concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	1.7 1.8	1.75	1.2 1.3	1.25	0.8 0.8	0.8	◉ ◉	◉	0 0	0	0 0	0
24	2.7 2.8	2.75	1.8 1.9	1.85	1.2 0.9	1.05	0.3 0.3	0.3	◉ ◉	◉	0 0	0
30	3.9 3.8	3.85	2.0 2.1	2.05	1.6 1.5	1.55	0.6 0.7	0.65	0.4 0.3	0.35	◉ ◉	◉
36	4.5 4.6	4.55	2.6 2.5	2.55	2.0 1.9	1.95	0.9 0.9	0.9	0.8 0.9	0.85	0.5 0.4	0.45
42	5.5 5.4	5.45	3.5 3.6	3.55	2.9 2.8	.85	1.3 1.2	1.25	1.0 1.1	1.05	0.8 0.9	0.85

Note : 0 indicates no growth
◉ indicates some growth

Table-23

Effect of sterilized P_0 concentration of fruit extract of *Phyllanthus emblica* Linn. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of sterilized P_0 concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	1.7 1.8	1.75	1.5 1.6	1.55	1.2 1.1	1.15	0.4 0.4	0.4	◉ ◉	◉	0 0	0
24	2.7 2.8	2.75	2.0 2.1	2.05	1.5 1.4	1.45	0.7 0.7	0.7	0.3 0.4	0.35	◉ ◉	◉
30	3.9 3.8	3.85	2.8 2.7	2.75	1.8 1.9	1.85	1.1 1.1	1.1	0.8 0.7	0.75	0.5 0.6	0.55
36	4.5 4.6	4.55	3.0 3.0	3.0	2.5 2.6	2.55	1.5 1.5	1.5	1.0 0.9	0.95	0.8 0.9	0.85
42	5.5 5.4	5.45	3.9 3.9	3.9	3.5 3.3	3.4	1.9 1.9	1.9	1.6 1.5	1.55	1.2 1.2	1.2

Note : 0 indicates no growth
◉ indicates some growth

Table-24

Effect of non-sterilized P_1 concentration of fruit extract of *Phyllanthus emblica* Linn. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of non-sterilized P_1 concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	1.7 1.8	1.75	1.5 1.6	1.55	1.0 1.2	1.1	0.3 0.4	0.35	◉ ◉	◉	0 0	0
24	2.7 2.8	2.75	1.9 1.9	1.9	1.5 1.4	1.45	0.8 0.6	0.7	0.5 0.5	0.5	◉ ◉	◉
30	3.9 3.8	3.85	2.2 2.1	2.05	1.8 1.9	1.85	0.9 0.8	0.85	0.7 0.7	0.7	◉ ◉	◉
36	4.5 4.6	4.55	2.5 2.6	2.55	2.0 2.2	2.1	1.3 1.5	1.4	1.0 1.1	1.05	0.8 0.7	0.75
42	5.5 5.4	5.45	3.9 4.0	3.95	2.9 3.0	2.95	1.8 1.9	1.85	1.5 1.5	1.5	1.1 1.2	1.15

Note : 0 indicates no growth
◉indicates some growth

Table-25

Effect of sterilized P_1 concentration of fruit extract of *Phyllanthus emblica* Linn. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of sterilized P_1 concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	1.7 1.8	1.75	1.8 1.7	1.75	1.3 1.4	1.35	0.8 0.7	0.75	0.3 0.3	0.3	◉ ◉	◉
24	2.7 2.8	2.75	2.2 2.0	2.1	1.8 1.9	1.85	0.9 1.1	1.0	0.7 0.6	0.65	0.5 0.4	0.45
30	3.9 3.8	3.85	2.4 2.3	2.35	2.0 2.1	2.05	1.2 1.2	1.2	0.9 1.2	1.05	0.8 0.7	0.75
36	4.5 4.6	4.55	2.8 2.9	2.85	2.5 2.4	2.45	1.7 1.8	1.75	1.5 1.4	1.45	1.2 1.2	1.2
42	5.5 5.4	5.45	4.2 4.5	4.35	3.1 3.2	3.15	2.0 2.1	2.05	1.9 1.9	1.9	1.6 1.5	1.55

Note : ◉ indicates some growth

Table-26

Effect of non-sterilized P_2 concentration of fruit extract of *Phyllanthus emblica* Linn. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of non-sterilized P_2 concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	1.7 1.8	1.75	1.3 1.3	1.3	0.5 0.6	0.155	0.6 0.4	0.65	0 0	0 0	0 0	0
24	2.7 2.8	2.75	1.8 1.7	1.75	1.2 0.9	0.7	0.7 0.9	0.8	0 0	0	0 0	0
30	3.9 3.8	3.85	2.8 2.9	2.85	1.5 1.6	1.55	1.2 1.3	1.25	⊙ ⊙	⊙	⊙ ⊙	⊙
36	4.5 4.6	4.55	3.5 3.4	3.45	2.0 1.9	1.95	1.8 1.9	1.85	0.5 0.6	0.55	0.3 0.4	0.35
42	5.5 5.4	5.45	4.6 4.9	4.75	2.5 2.7	2.6	2.2 2.3	2.25	0.9 0.9	0.9	0.7 0.8	0.75

Note : 0 indicates no growth
⊙ indicates some growth

Table-27

Effect of sterilized P_2 concentration of fruit extract of *Phyllanthus emblica* Linn. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of sterilized P_2 concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	1.7 1.8	1.75	1.5 1.7	1.6	1.3 1.4	1.35	1.2 0.9	1.05	0.5 0.5	0.5	0 0	0
24	2.7 2.8	2.75	1.9 1.9	1.9	1.7 1.8	1.75	1.3 1.4	1.35	0.8 0.9	0.85	00.3 0.3	0.3
30	3.9 3.8	3.85	3.1 3.1	3.1	2.2 2.1	2.15	1.8 1.7	1.75	1.1 1.1	1.1	0.8 0.7	0.75
36	4.5 4.6	4.55	3.8 3.7	3.75	2.4 2.4	2.4	2.3 2.4	2.35	1.3 1.4	1.35	0.9 0.9	0.9
42	5.5 5.4	5.45	4.8 5.0	4.9	3.0 2.9	2.95	2.5 2.6	2.55	1.6 1.7	1.67	1.2 1.3	1.25

Note : 0 indicates no growth

Table-28

Effect of non-sterilized P_3 concentration of fruit extract of *Phyllanthus emblica* Linn. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of non-sterilized P_3 concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	1.7 1.8	1.75	1.9 1.8	1.85	1.5 1.6	1.55	0.8 0.9	0.85	0.5 0.4	0.45	◉ ◉	◉
24	2.7 2.8	2.75	2.5 2.4	2.45	2.0 2.0	2.0	1.3 1.2	1.25	0.7 0.6	0.65	0.5 0.6	0.55
30	3.9 3.8	3.85	2.9 2.8	2.85	2.4 2.3	2.35	1.8 1.8	1.8	1.1 1.1	1.1	0.9 0.9	0.9
36	4.5 4.6	4.55	3.2 3.0	3.1	2.7 2.9	2.8	2.7 2.6	2.65	2.3 2.4	2.35	1.5 1.4	1.45
42	5.5 5.4	5.45	4.5 4.6	4.55	3.6 3.5	3.55	3.0 2.9	2.95	2.8 2.6	2..7	1.9 1.7	1.8

Note : ◉ indicates some growth

Table-29

Effect of sterilized P_3 concentration of fruit extract of *Phyllanthus emblica* Linn. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of sterilized P_3 concentration											
	o drop (control)		1 drop		2 drops		0.5 ml		1 ml		2 ml	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
18	1.7 1.8	1.75	1.9 1.9	1.65	1.7 1.6	1.15	1.0 1.0	1.0	0.6 0.6	0.6	0.3 0.3	0.3
24	2.7 2.8	2.75	2.6 2.7	2.65	2.3 2.4	2.35	1.5 1.6	0.155	0.8 0.9	0.85	0.7 0.8	0.75
30	3.9 3.8	3.85	3.0 3.2	3.1	2.9 2.9	2.9	2.5 2.5	2.5	1.2 1.2	1.2	1.0 0.9	0.95
36	4.5 4.6	4.55	3.9 3.7	3.8	3.3 3.5	3.4	2.8 2.9	2.85	2.5 2.7	2.6	1.8 1.7	1.75
42	5.5 5.4	5.45	5.0 5.0	5.0	4.3 4.6	4.45	3.4 3.4	3.4	2.9 3.0	2.95	2.0 2.2	2.1

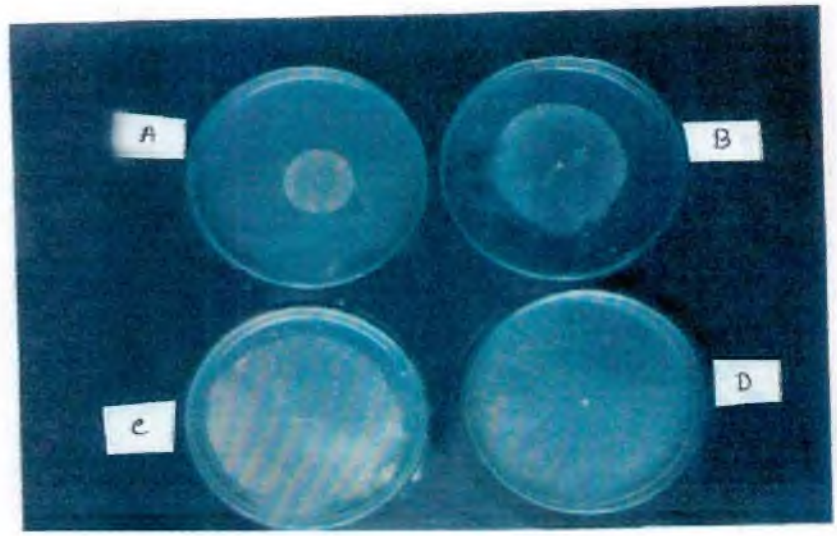


Fig:3. Effect of different doses of non- sterilized Po concentration of *Phyllanthus emblica* Linn. on the growth of *Neurospora crass.* **A**.0.5 ml, **B**.2drops **C**. 1 drop and **D**. 0 drop (control).

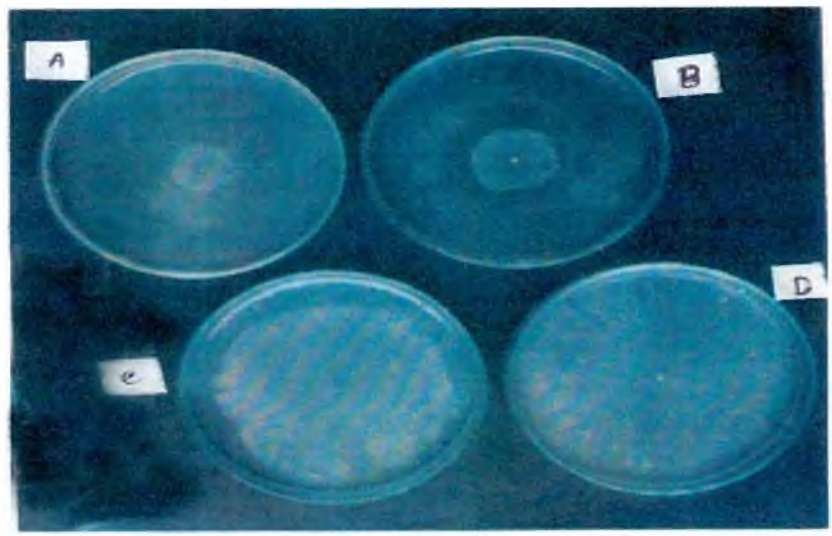


Fig: 4. Effect of different doses of non- sterilized P_2 concentration of *Phyllanthus emblica* Linn. on the growth of *Neurospora crass.* **A**.0.5 ml, **B**.2drops **C**. 1 drop and **D**. 0 drop (control).

Conclusion: From table 22,23,24,25,26,27,28 and 29 it was found that, the concentration of fruit extract of *phyllanthus emblica* Linn. control the radial growth of wild type (Ema) *Neurospora crassa*. The result also showed that non-sterilized concentration was more effective than sterilized one. Non-sterilized P_2 concentration was thee most effective and sterilized P_3 concentration was the lest effective.

So,non-sterilized P_0 and P_2 concentration of fruit extract of *phyllanthus emblica* Linn. were selected for treating wild type (Ema) conidia.

5.4.2 STUDY OF MUTAGENIC EFFECT OF FRUIT EXTRACT OF *Phyllanthus emblica* Linn. IN *Neurospora crassa.*

Fruit extract of different concnetrations were used to test the mutagenic affect on *Neurospora crassa*. Certain concentrations were effective. These concentrations had appropriate mutagenic effective *Ncurospora crassa*.

5.4.3 INDUCTION OF MUTATION WITH FRUIT EXTRACT (*Phyllanthus emblica* Linn.) SOLUTION OF THE CONCENTRATION P_0 AND P_2.

Conidia of Ema of 5 days old culture was treated with fruit extract *(Phyllanthus emblica* Linn) concentration of P_o and P_2 for 10 minutes. SM was used for plating and VM in small tubes was used as culturing media. Plates with treated conidia were incubated at 25^0C. Single conidial, colonies were isolated after 48 hours of incubation.

Then the cultures were incubated at 25^0C and observed after 5 days. The cultures that shows abnormality with Ema of same age were denoted as mutants. The mutants were different from Ema in colour of conidia, mode of conidia, mycelial structure etc.

5.4.4 CLASSIFICATION AND NOMENCLATURE OF OBTAINED MUTANTS INDUCED WITH FRUIT EXTRACT of *Phyllanthus emblica* Linn.

Group – A : albino

Less growth, mycelia very scantry in number, conidia completely colourless.

The following 10 mutants fall into this group.

$P_0$705, $P_0$713, $P_0$726, P_o728, P_o750, P_o758, $P_2$800, $P_2$825 ,$P_2$919, $P_2$955.

Group – B : checked

Very checked growth, mycelia more or less colourless, hypae very small, growth smaller than the wild, conidia not purely orange in colouration.

The following 12 mutants fall into this group.

$P_0$738, $P_0$739, $P_0$762, P_o765, P_o769, $P_2$809, $P_2$813 ,$P_2$862,$P_2$875. P931, $P_2$932, $P_2$960.

Group – C: fluffy

The mycelial growth more than wild type while the conidial growth is very much less than wild type. The mycelia are cottony and compactly arranged.

The following 13 mutants fall into this group.

$P_0$782, $P_0$785, $P_0$787, P_o791, P_o795, $P_0$827, $P_2$838 ,$P_2$940,$P_2$945. P986, $P_2$1009, $P_2$1017. $P_2$1027.

Group – D : conidial band

Densed conidial growth formed a band at the tip of the growth. Conidia are light orange or pink.

The following 14 mutants fall into this group.

$P_0$713, $P_0$717, $P_0$729, P_o732, P_o734, P_o770, $P_2$803, $P_2$807, $P_2$861,$P_2$990. $P_2$997, $P_2$998, $P_2$1032 $P_2$1049.

Group – E : buff

buff coloured mycelia and conidia, growth on the surface of the media, growth less than wild .

The following 2 mutants fall into this group.

$P_2$1015, $P_2$1024.

Group – F ropy

The hypae look like beautiful ropes, conidia pinkish orange, growth more or less checked

The following 9 mutants fall into this group.

$P_0$910, $P_0$921, $P_0$942, $P_0$980, $P_2$1060, $P_2$1069, $P_2$1074, $P_2$1078, $P_2$1090

Table-30

Classification of mutants induced with fruit extract of *Phyllanthus emblica* Linn.

Name of the group	Characteristics of the mutants	Concentration of the fruit extract of ***Phyllanthus emblica* Linn.**	Number of the mutants	Name of the mutants
A	Both mycelia and conidia are colour less	P_0 P_2	6 4	albino
B	Growth less than wild type	P_0 P_2	5 7	checked
C	profuse mycelial growth and no conidiation	P_0 P_2	6 7	fluffy
D	Conidia formed a band at the tip	P_0 P_2	6 8	conidial band
E	Buff colour mycelia and conidia present on the surface of the tube	P_2	2	buff
F	Mycelia rope like	P_0 P_2	4 5	ropy

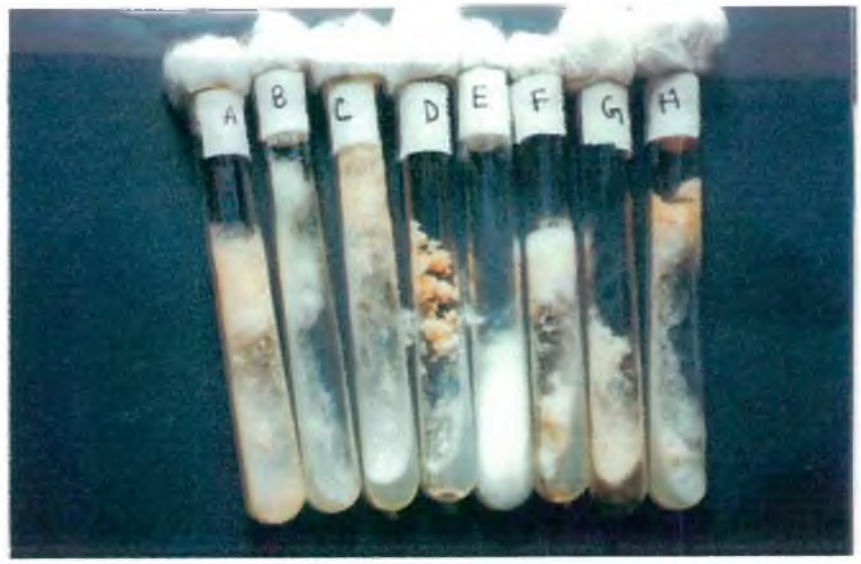


Fig : 5. Comparative morphological features of **A.** Ema (wild type), **B.**fl. (U1520), **C.**vg. ($A_1$550) **D.** ro (A_o307) **E.** al ($P_2$919). **F.** fluffy. band ($C_1$149). **G.** ch (Ao380), **H.** clf. ($A_1$415).

Fig : 6. Comparative morphological features of Ema (wild type), **A** . Con. band ($P_0$729), **B.**fl. band ($C_1$149), **C**. ro(P_o910), **D**. mat (C_o65), **E**. al ($P_2$919), **F**. cr ($A_1$515).

5.5 Detailed study of some selected mutants induced with leaf extract of *Adhatoda zeylanica* Nees, leaf extract of *Centella asiatica* (Linn) Urban and Fruit extract of *Phyllanthus emblica* Linn.

Table-31

The following 12 mutants were obtained from leaf extract of *Ahatoda zeylanica* Nees, leaf extract of *Centella asiatica* (Linn) Urban and Fruit extract of *Phyllanthus emblica* Linn.

No. of the mutant	Name of the mutants
A_0307	ropy
A_o380	checked
A_1415	cauliflower
A_1550	vigorous
A_1515	crescent
C_o20	plug
C_o65	mat
C_1149	fluffy band
P_o729	conidial band
P_2919	albino
P_2945	fluffy
P_21015	buff

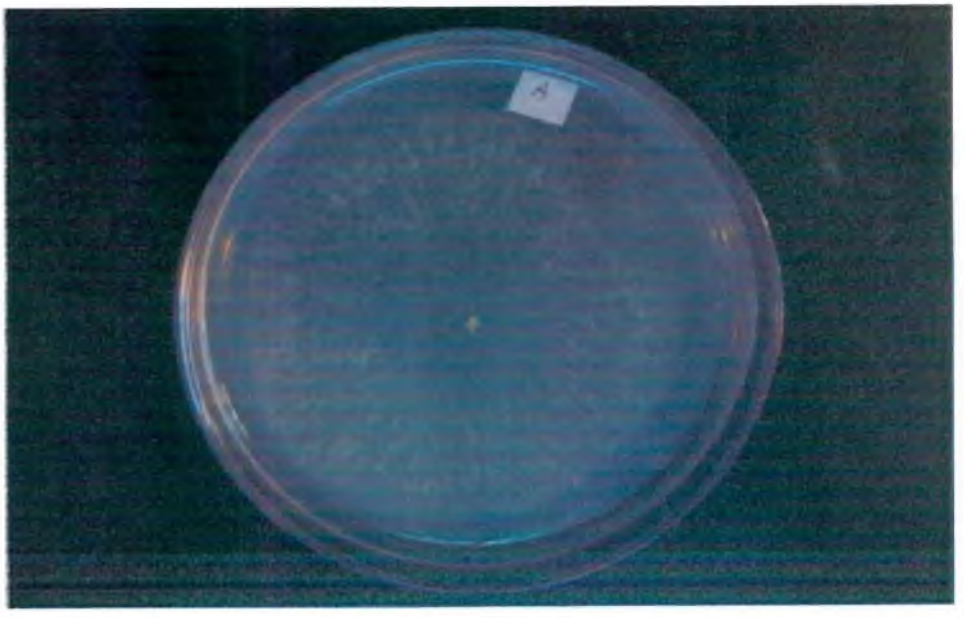


Fig : 7. Colonial feature of fluffy (P_2945).

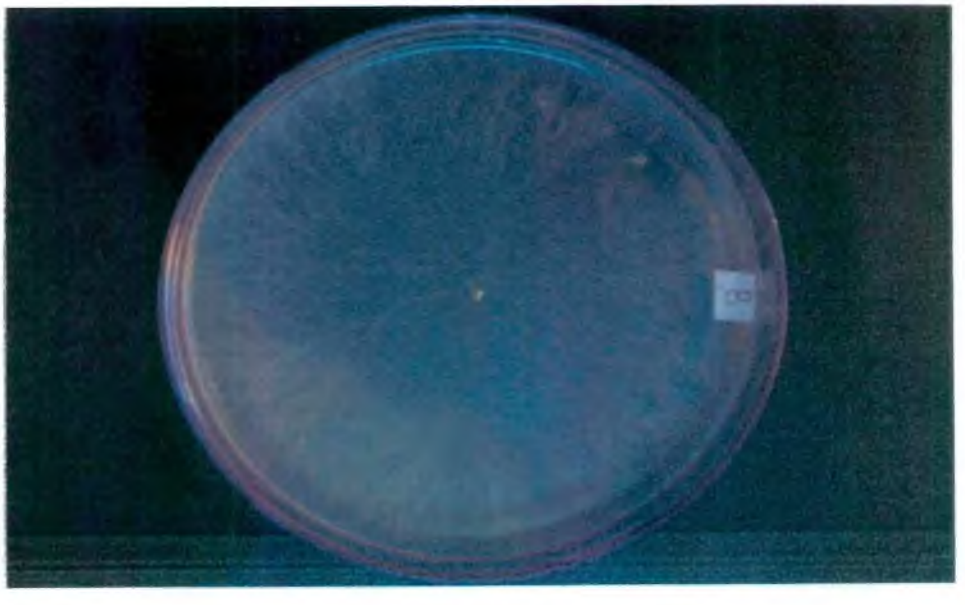


Fig : 8. Colonial feature of Ema (wild).

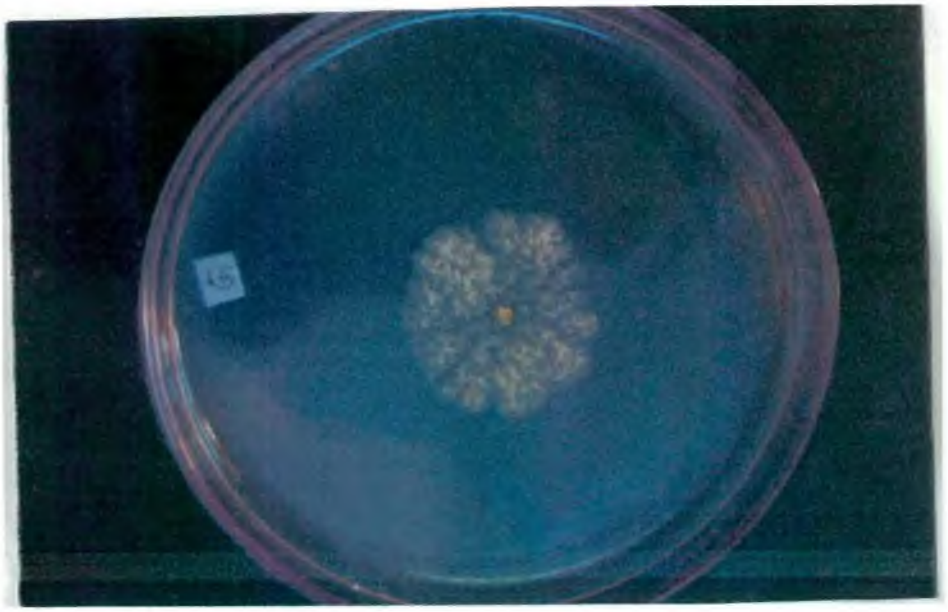


Fig : 9. Colonial feature of ropy (A_o307).

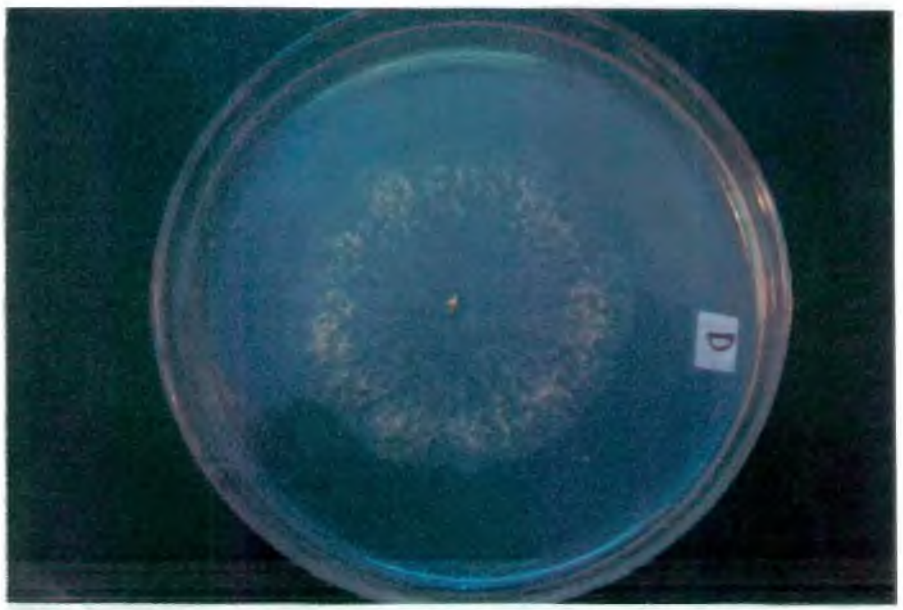


Fig: 10. Colonial feature of con. band (P_o729).

Fig: 11. Colonial feature of vigorous (A_1550).

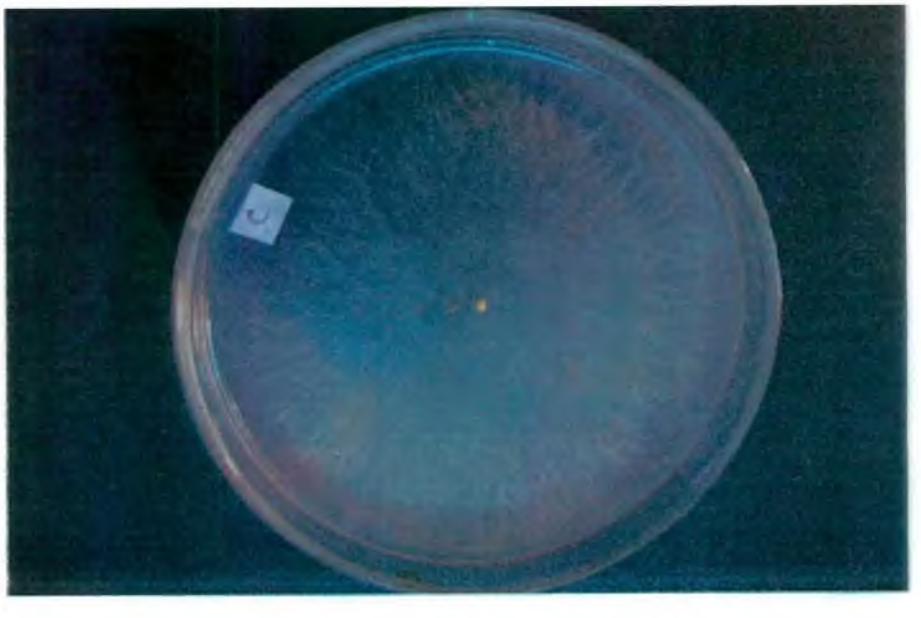


Fig : 12. Colonial feature of yellow fluffy band (C_1149).

Table-32

Time required for germination of Ema and some selected mutants induced with leaf extract of *Adhatoda zeylanica* Nees, *Centella asiatica* (Linn) Urban and fruit extract of *phyllanthus emblica* Linn

Name of the culture	Observation after the following hours							Inference on germination
	1	2	3	4	5	6	7	
Ema	Germination not started	Germination not started	Germination just started	Clear germination observed	Germination completed			Eama germinates with in 5 hours
ropy (ao-307)	Germination not started	Germination not started	Germination not started	Germination just stared	Clear Germination observed	Germination completed		ropy germinates with in 6 hours
albino (P2-919)	Germination not started	Germination not started	Germination not started	Germination not stared	conidia became swollen	Germination just started	Germinati-on completed	albinogerminates with in 7 hours
conidial band (Po-729)	Germination not started	Germination not started	Germination not started	Germination just stared	Germination completed			conidial band germinates with in 5 hours
Checked (Ao-380)	Germination not started	Germination not started	Germination not started	conidia became swollen	Germination just started	Germination completed		checkedgerminates with in 6 hours
Vigorous (A1-550)	Germination not started	Germination not started	Germination not started	Germination just stared	Germination completed			vigorousgerminates with in 5 hours
plug (Co-20)	Germination not started	Germination not started	Germination just started	Germination completed				plug germinates with in 4 hours
fluffy band (C1-149)	Germination not started	Germination not started	Germination not started	Germination just started	Germination completed			fluffy band germinates with in 5 hours
mat (Co-65)	Germination not started	Germination not started	Germination not started	Germination not stared	conidia became swollen	Germination just started	Germination completed	mat germinates with in 7 hours

(Contd...)

Table-32 (Contd.)

Name of the culture	Observation after the following hours							Inference on germination
	1	2	3	4	5	6	7	
cauliflower ($A_1$415)	Germination not started	Germination not started	Germination not started	Conidia become swollen	Germination just started	Clear germinatio-n observed	Germinati-on completed	cauliflower germinates with in 7 hours
fluffy ($P_2$945)	Germination not started	Germination not started	Germination not started	Clear germination observed	Germination completed			fluffy germinates with 5 hours
cresent ($A_1$515)	Germination not started	Germination not started	Germination not started	Germination just stared	Clear germination observed	Germinati-on completed		crescent germinates with in 6 hours
buff ($P_2$1015)	Germination not started	Germination not started	Germination not started	Conidia become swollen	Germination observed	Germinati-on completed		buff germinates with in 6 hours

Conclusion: The mutants showed variation in their time required for germination with that of wild type Ema

Table-33

Radial growth of Ema and some selected induced with leaf extract of *Adhatoda zeylanica* Nees, *Centella asiatica* (Lim) Urban and fruit extract of *Phyllanthus emblia* Linn.

Name of the culture	Observation after the following hours											
	18		24		30		36		42		48	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
Ema	1.5 1.6	1.55	2.0 2.1	2.05	2.5 2.6	2.55	3.0 3.1	3.05	3.4 3.5	3.45	3.9 3.8	3.85
plug (Co-20)	2.0 2.1	2.05	3.2 3.4	3.3	3.8 3.10	3.45	4.2 4.1	4.15	4.6 4.5	4.55	5.2 5.1	5.15
vigorus A_1-550	1.9 1.8	1.85	2.5 2.6	2.55	2.9 2.11	2.505	3.6 3.7	3.65	4.0 4.0	4.0	4.5 4.4	4.45
fluffy band (C_1-149)	1.7 1.7	1.7	2.4 2.6	2.5	2.8 2.9	2.85	3.5 3.6	3.55	3.9 3.9	3.9	4.4 4.3	4.35
con. band (Po-729)	1.7 1.6	1.65	2.3 2.1	2.2	2.6 2.5	2.55	3.5 3.5	3.5	3.8 3.7	3.75	4.0 4.1	4.05
ropy (A_0-307)	0.8 0.6	0.7	1.8 1.9	1.85	2.3 2.2	2.25	2.8 2.9	2.85	3.4 3.3	3.35	3.9 3.8	3.85
checked (A_0-380)	0.5 0.4	0.45	1.5 1.5	1.5	1.8 1.10	1.45	2.3 2.2	2.25	2.7 2.6	2.65	3.3 3.2	3.25
albino (P_2-919)	0.3 0.4	0.35	1.5 1.3	1.4	1.8 1.7	1.75	2.0 1.9	1.95	2.4 2.3	2.35	2.8 2.9	2.85
mat (Co-65)	0.2 0.3	0.25	1.3 1.1	1.2	16. 1.5	1.55	1.9 1.8	1.85	2.0 2.1	2.05	2.5 2.4	2.45

(Contd...)

Table-33 (Contd.)

Name of the culture	Observation after the following hours											
	18		24		30		36		42		48	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
crescent ($A_1$515)	0.5 0.6	0.55	1.5 1.4	1.45	2.0 2.1	2.05	2.3 2.5	2.4	3.4 3.3	3.35	3.8 3.7	3.75
cauliflower ($A_1$415)	1.3 1.4	1.35	1.6 1.7	1.65	2.2 2.4	2.3	2.8 2.7	2.75	3.5 3.4	3.45	3.9 3.8	3.85
fluffy ($P_2$945)	0.5 0.6	0.55	1.5 1.6	1.55	2.1 2.2	2.15	2.7 2.8	2.75	3.0 3.1	3.05	3.5 3.6	3.55
buff ($P_2$1015)	0.6 0.6	0.6	1.8 1.7	1.75	2.0 2.0	2.0	2.5 2.6	2.55	2.8 2.9	2.85	3.5 3.5	3.5

Conclusion : The mutants showed variation in their radial growth with that of wild type Ema.

Table-34

Linear growth of Ema and some selected mutants induced with leaf extract of *Adhatoda zeylanica* Nees. *Centella asiatica* (Linn.) Urban and fruit extract of *Phyllanthus emblica* Linn.

Name of the culture	Observation after the following hours													
	18		24		30		36		42		48		54	
	growth in cm	mean in cm	growth in cm	mean in cm	growth in cm	mean in cm	growth in cm	mean in cm	growth in cm	mean in cm	growth in cm	mean in cm	growth in cm	mean in cm
Ema	2.5 2.4	2.45	3.0 3.3	3.15	3.5 3.6	3.55	4.4 4.2	4.3	5.0 5.1	5.05	5.8 5.7	5.75	6.3 6.2	6.25
plug (Co 20)	3.0 3.2	3.1	3.5 3.8	3.65	4.3 4.2	4.25	5.2 5.1	5.15	6.0 6.2	6.1	7.5 7.7	7.6	8.4 8.5	8.45
vigorus (A_1 550)	2.8 2.7	2.75	3.3 3.4	3.35	3.7 3.5	3.6	4.6 4.5	4.55	5.5 5.4	5.45	6.2 6.1	6.15	6.8 6.9	6.85
fluffy band (C_1 149)	2.6 2.4	2.5	3.2 3.1	3.15	3.5 3.4	3.45	4.4 4.5	4.35	5.2 5.1	5.15	5.8 5.7	5.75	6.5 6.4	6.45
conidial band (Po-729)	2.3 2.1	2.2	2.8 2.7	2.75	3.0 2.9	2.95	3.5 3.2	3.35	4.6 4.5	4.55	5.5 5.6	5.55	6.0 6.2	6.1
ropy (A_0 307)	1.5 1.6	1.55	2.3 2.4	2.35	2.6 2.7	2.65	3.0 3.1	3.05	3.9 3.9	3.9	4.8 4.9	4.85	5.6 5.4	5.5
Checked (A_0-380)	0.9 0.8	0.85	1.2 1.0	1.1	1.9 1.8	1.85	2.5 2.6	2.55	3.3 3.2	3.25	3.9 3.8	3.85	4.2 4.1	4.15
albino (P_2 919)	0.5 0.4	0.45	0.9 0.7	0.8	1.5 1.4	1.45	2.4 2.2	2.3	2.8 2.7	2.75	3.0 3.0	3.0	3.5 3.4	3.45
mat (C_0 65)	0.4 0.2	0.3	0.8 0.6	0.7	1.3 1.2	1.25	1.7 1.8	1.75	2.0 2.1	2.05	2.3 2.4	2.35	2.8 2.7	2.75

(Contd.....)

Table-34 (Contd..)

Name of the culture	Observation after the following hours													
	60		66		72		78		84		90		96	
	growth in cm	mean in cm	growth in cm	mean in cm	growth in cm	mean in cm	growth in cm	mean in cm	growth in cm	mean in cm	growth in cm	mean in cm	growth in cm	mean in cm
Ema	9.9 9.5	9.7	11.7 12.0	11.58	13.8 14.1	13.95	16.2 16.8	16.5	19.5 20.1	19.8	22.8 22.7	22.75	25.5 26.0	25.75
plug (C_0 20)	10.8 11.2	11	13.5 13.9	13.7	17.6 18.1	17.85	21.4 22.2	21.8	25.6 25.9	25.75	29.6 30.1	29.85	33.6 34.3	33.95
vigorus (A_1 550)	8.9 9.0	8.95	10.8 11.2	11	12.2 12.7	12.45	14.4 14.8	14.2	16.8 17.0	16.9	19.5 19.8	19.65	21.9 22.3	22.1
fluffy band (C_1 149)	8.7 8.5	8.6	9.8 10.0	9.9	11.3 11.6	11.45	13.2 13.5	13.35	14.8 15.2	15	16.0 16.9	16.45	18.2 18.6	18.4
con. band (P_0 729)	7.3 7.9	7.6	8.5 8.9	8.7	9.2 9.6	9.4	10.6 11.0	10.8	12.5 12.9	12.7	13.3 13.7	13.5	14.5 14.8	14.65
ropy (A_0 307)	6.5 6.8	6.65	7.3 7.5	7.4	7.8 7.9	7.85	8.2 8.5	8.35	8.6 8.9	8.75	9.6 9.8	9.7	10.0 10.2	10.1
Checked (A_0 380)	5.6 5.7	5.65	6.5 6.9	6.7	7.6 8.0	7.8	8.5 8.2	8.35	8.7 8.4	8.55	9.0 9.0	9.0	9.4 9.5	9.45
albino (P_2 919)	4.0 4.1	4.05	4.5 4.5	4.5	5.2 5.3	5.25	5.7 5.9	5.8	6.1 6.2	6.15	6.6 6.7	6.65	7.3 7.5	7.4
mat (C_0 65)	3.0 3.3	3.15	3.5 3.9	3.7	4.2 4.7	4.45	4.8 5.0	4.9	5.2 5.7	5.45	5.6 6.0	5.8	6.2 6.5	6.36

Conclusion : The linear growth of the mutants were different from that of wild type Ema.

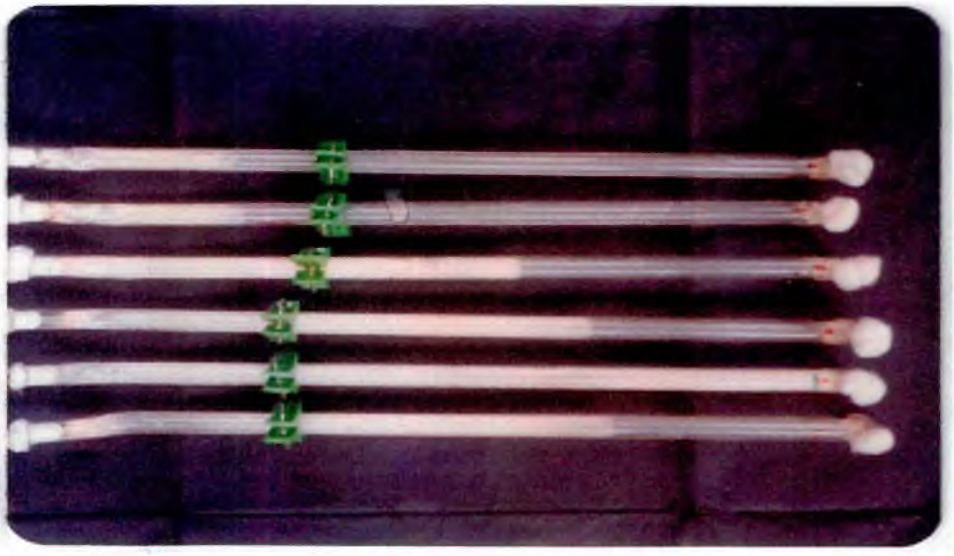


Fig:13. Linear growth of Ema (wild type), ropy (A_o307), clf. ($A_1$415),con. band (P_o729), vig.(A_1-550)fl.($P_2$945) after 82 hours . (up to down).

Table –35

Dry mycelial weight of Ema and some selected mutants induced with leaf extract of *Adhatoda zeylanica* Nees., *Centella asiatica* (Linn.) Urban and fruit extract of *Phyllanthus embica* Linn. after 72 hours.

Number and name of the culture	Weight of the empty filter paper A	Weight of the filter paper containing dried mycelia (g) B	Dry weight of the mycelia (g) B-A	Mean (g)	Inference on mycelial growth
Ema	0.7573 0.7573	0.8680 0.8774	0.1107 0.1201	0.1154	Normal
ropy (Ao-307)	0.7656 0.7668	0.8731 0.8701	0.1075 0.1033	0.1054	Growth less than normal
albino (P2-919)	0.7740 0.7796	0.8626 0.8599	0.0886 0.0803	0.08445	Growth less than normal
Con. band (P_o-729)	0.7538 0.7475	0.8853 0.8812	0.1315 0.1337	0.1326	Growth more than normal
checked (A_o-380)	0.7538 0.7589	0.8899 0.8874	0.1361 0.1285	0.1323	Growth more than normal
vigorous (A_I-550)	0.7650 0.7618	0.8816 0.8812	01166 0.1194	0.1180	Growth more than normal
plug (Co-20)	0.7435 0.7440	0.8825 0.8795	0.1390 0.1355	0.13725	Growth more than normal
fluffy (C_I-149)	0.7669 0.7616	0.8990 0.8912	0.1321 0.1296	0.13085	Growth more than normal
mat (C_o-65)	0.7589 0.7589	0.8623 0.8608	0.1034 0.1019	0.10265	Growth less than normal

(Contd....)

Table-35 (contd.)

Number and name of the culture	Weight of the empty filter paper (g)	Weight of the filter paper containing dried mycelia (g) B	Dry weight of the mycelia (g) B-A	Mean (g)	Inference on mycelial growth
cauliflower ($A_1$415)	0.7527 0.7530	0.8800 0.8680	0.1273 0.1150	0.12115	Growth less than normal
buff ($P_2$1015)	0.7519 0.7512	0.8840 0.8805	0.1321 0.1293	0.1307	Growth less than normal
fluffy ($P_2$945)	0.7565 0.7616	0.8625 0.8686	0.1060 0.1070	0.1065	Growth less than normal
crescent ($A_1$515	0.7523 0.7532	0.8830 0.8868	0.1307 0.1336	0.13215	Growth less than normal

Conclusion : The mutants showed variation in their mycelial growth with wild type Ema.

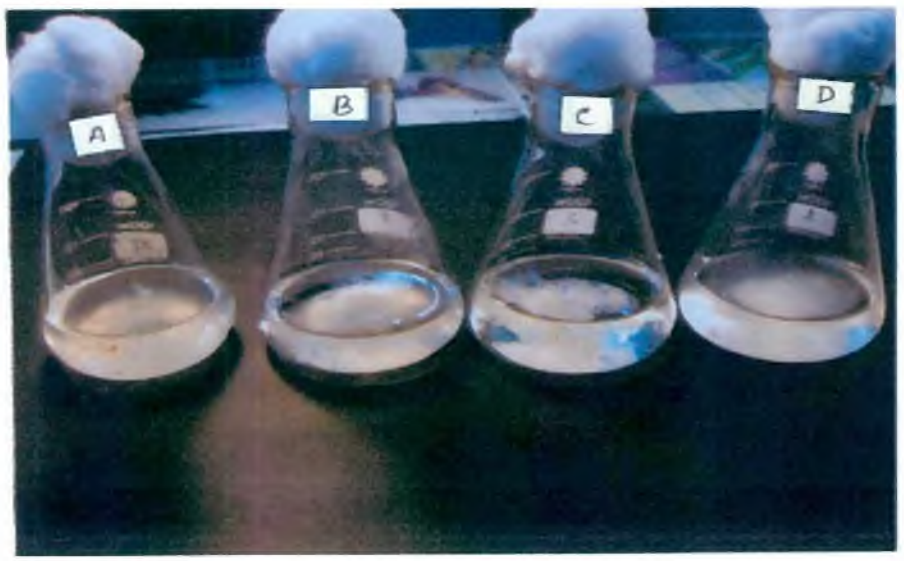


Fig: 14. Mycelial growth of **A**. Ema (wild type) and some mutants **B**. ropy (A_o 307), **C**. con. band (P_o729), **D**.vig. ($A_1$550), after 72 hours.

Fig:15. Mycelial growth of some mutants **A**. albino ($P_2$919), **B**. checked (A_o380), **C**. fluffy band ($C_1$149), **D**. mat (C_o65) after 72 hours.

Table – 36

Fertility and mating types of some selected mutants induced with leaf extract of *Adhatoda zeylanica* Nees, *Centella asiatica* (Linn.) Urban and fruit extract of *Phyllanthus emblica* Linn.

Name of the groups	Name of the cross	Perithecia formed in days	Size and no. of perithecia	Spore sheddi-ng in days	Fertility	Mating type
ropy	$A_0$307 × Ema	-	-	-	-	'a'
	$A_0$307 × EmA	5	Large and many	15	Good fertile	
	$A_1$424 × Ema	-	-	-	-	
	$A_1$424 × EmA	8	Medium and many	14	Good fertile	'a'
ckecked	$A_0$380 × Ema	-	-	-	-	'a'
	$A_0$380 × EmA	6	Medium and many	15	Good fertile	
	$A_1$465 × Ema	-	-	-	-	
	$A_1$465 × EmA	9	Large and many	17	fertile	'a'
vigorus	$A_0$365 × Ema	-	-	-	-	'a'
	$A_0$365 × EmA	8	Medium and many	18	Fertile	
	$A_1$550 × Ema	-	-	-	-	
	$A_1$550 × EmA	7	Large and many	14	Good fertile	'a'
plug	$C_0$20 × Ema	-	-	-	-	'a'
	$C_0$20 × EmA	8	Medium and many	18	Fertile	
	$C_1$125 × Ema	-	-	-	-	
	$C_1$125 × EmA	9	Medium and 15-16	19	Less fertile	'a'
fluffy band	$C_0$60 × Ema	-	-	-	-	'a'
	$C_0$60 × EmA	8	Medium and some	19	Fertile	
	$C_1$149 × Ema	-	-	-	-	
	$C_1$149 × EmA	8	Small and many	21	Fertile	'a'
mat	$C_0$65 × Ema	-	-	-	-	'a'
	$C_0$65 × EmA	12	Medium and few	21	Less fertile	
	$C_1$150 × Ema	-	-	-	-	
	$C_1$150 × EmA	12	Small and 10-15	28	Less fertile	'a'

(contd.....)

Table – 36 (Contd..)

Name of the groups	Name of the cross	Perihelia formed in days	Size and no of perihelia	spore sheddin-g in days	Fertility	Matin g type
albino	$P_0$726 × Ema	-	-	-	-	'a'
	$P_0$726 × EmA	12	Small and few	28	Less fertile	
	$P_2$919 × Ema	-	-	-	-	
	$P_2$919 × EmA	14	Medium and many	28	Less fertile	'a'
conidial band	$P_0$729 × Ema	-	-	-	-	'a'
	$P_0$729 × EmA	7	Medium and many	16	Good fertile	
	$P_2$803 × Ema	-	-	-	-	
	$P_2$803 × EmA	7	Large and many	16	Good fertile	'a'
cauliflower	$A_1$415 × Ema	-	-	-	-	'a'
	$A_1$415 × EmA	7	Medium and many	13	Good fertile	
	$A_1$415 × Ema	-	-	-	-	
	$A_1$415 × EmA	7	Medium and many	13	Good fertile	'a'
crescent	$A_1$515 × Ema	-	-	-	-	'a'
	$A_1$515 × EmA	7	Large and several	17	Less fertile	
	$A_1$515 × Ema	-	-	-	-	
	$A_1$515 × EmA	8	Medium and serveral	18	Less fertile	'a'
buff	$P_2$1015 × Ema	-	-	-	-	'a'
	$P_2$1015 × EmA	9	Medium and many	17	fertile	
	$P_2$1015 × Ema	-	-	-	-	
	$P_2$1015 × EmA	9	Small and many	16	Fertile	'a'
fluffy	$P_2$945 × Ema	-	-	-	-	'a'
	$P_2$945 × EmA	5	Large and many	13	Good fertile	
	$P_2$945 × Ema	-	-	-	-	
	$P_2$945 × EmA	6	Medium and many	14	Good fertile	'a'

Table – 37

Segregation ratio of some selected mutants induced with leaf extract of *Adhatoda zeylanica* Nees, *Centella asiatica* (Linn.) Urban and fruit extract of *phyllanthus emblica* Linn.

Name of the cross	No. of spores isolated	No. of wild	No. of mutant	Segregation ratio (wild: mutant)
ropy	72	32	40	1 : 1.25
checked	90	44	46	1 : 1.05
vigorus	90	41	49	1 :1.20
Plug	85	40	45	1 : 13
fluffy band	100	49	51	1 : 1.04
mat	80	39	41	1 : 1.05
albino	75	35	40	1 : 1.14
conidial band	110	55	55	1 : 1
cauliflower	90	43	47	1 : 1.09
crescent	85	41	44	1 : 1.07
buff	77	32	45	1 : 1.41
fluffy	100	48	52	1 : 1.08

Fig: 16. A fertile cross showing huge mature perithecia

5.6 PREPARATION OF EXTRACT OF SOME MUTANTS FOR THE STUDY OF LINKAGE.

For the preparation of extract and for study of linkage, the mutants were crossed with the respresentatives of different linkage groups. Westergaard's crossing medium was supplemented with necessary amino-acid (20 mg for 100 ml media).

Table – 38

Result of the crosses of mutants and markers.

Name of the mutants	Name of the cross	Perithecia formed in days	Days taken for spore shedding	Size and number of perithecia	Linkage group of the marker	Fertility
ropy	$A_0$307 × I eu-3A	6	15	Large and many	I	Good fertile
	$A_0$307 × arg-5A	6	14	Large and many	II	Good fertile
	$A_0$307 × trp-1A	9	21	Medium and some	III	Fertile
	$A_0$307 × trp-4A	8	20	Small and many	IV	Fertile
	$A_0$307 × lysi-1A	9	24	Medium and some	V	Fertile
	$A_0$307 × trp-2A	7	13	Large and many	VI	Good fertile
	$A_0$307 × arg-10A	7	13	Large and many	VII	Good fertile
conidial band	$P_0$729 × I eu-3A	8	17	Large and many	I	Fertile
	$P_0$729 × arg-5A	8	18	Large and some	II	Fertile
	$P_0$729 × trp-1A	10	25	Small and some	III	Less Fertile
	$P_0$729 × trp-4A	8	15	Medium and many	IV	Good Fertile
	$P_0$729 × lysi-1A	9	19	Medium and some	V	Fertile
	$P_0$729 × trp-2A	7	14	Large and many	VI	Good fertile
	$P_0$729 × arg-10A	7	14	Large and many	VII	Good fertile

(contd....)

Table – 38 (contd..)

Name of the mutants	Name of the cross	Perithecia formed in days	Days taken for spore shedding	Size and number of perithecia	Linkage group of the marker	Fertility
cauliflower	$A_1$415 × I eu-3A	7	14	Medium and many	I	Good Fertile
	$A_1$415 × arg-5A	7	14	Medium and many	II	Good Fertile
	$A_1$415 × trp-1A	7	14	Small and some	III	Good Fertile
	$A_1$415 × trp-4A	8	16	Medium and many	IV	Fertile
	$A_1$415 × lysi-1A	9	21	Medium and few	V	Less Fertile
	$A_1$415 × trp-2A	8	21	Medium and some	VI	fertile
	$A_1$415 × arg-10A	8	15	Small and many	VII	Good fertile
crescent	$A_1$515 × I eu-3A	10	25	Small and 10-12	I	Less Fertile
	$A_1$515 × arg-5A	7	14	Large and many	II	Good Fertile
	$A_1$515 × trp-1A	7	14	Large and many	III	Good Fertile
	$A_1$515 × trp-4A	7	18	Medium and many	IV	Fertile
	$A_1$515 × lysi-1A	10	25	Medium 5-7	V	Less Fertile
	$A_1$515 × trp-2A	7	18	Medium and many	VI	Fertile
	$A_1$515 × arg-10A	9	18	Small and some	VII	Fertile
checked	$A_0$380 × I eu-3A	7	14	Medium and many	I	Good fertile
	$A_0$380 × arg-5A	7	14	Medium and many	II	Good fertile
	$A_0$380 × trp-1A	7	16	Small and some	III	Fertile
	$A_0$380 × trp-4A	8	18	Large and few	IV	Less fertile
	$A_0$380 × lysi-1A	9	20	Large and 5-7	V	Less fertile
	$A_0$380 × trp-2A	8	21	Medium and many	VI	Fertile
	$A_0$380 × arg-10A	8	21	Medium and many	VII	Fertile

(contd....)

Table – 38 (contd..)

Name of the mutants	Name of the cross	Perithecia formed in days	Days taken for spore shedding	Size and number of perithecia	Linkage group of the marker	Fertility
fluffy	$P_2$945 × I eu-3A	9	20	Medium and some	I	Fertile
	$P_2$945 × arg-5A	10	19	Medium and many	II	Fertile
	$P_2$945 × trp-1A	10	23	Medium and few	III	Less Fertile
	$P_2$945 × trp-4A	7	14	Large and many	IV	Good Fertile
	$P_2$945 × lysi-1A	8	17	Medium and many	V	Fertile
	$P_2$945 × trp-2A	7	15	Small and many	VI	Good fertile
	$P_2$945 × arg-10A	7	15	Small and many	VII	Good fertile
Vigorous	A550 × I eu-3A	7	14	Large and many	I	Good Fertile
	A550 × arg-5A	7	14	Medium and many	II	Good Fertile
	A550 × trp-1A	9	19	Medium and few	III	Less Fertile
	A550 × trp-4A	7	18	Medium and many	IV	Fertile
	A550 × lysi-1A	8	19	Small and some	V	Fertile
	A550 × trp-2A	9	21	Large and 15-20	VI	Less fertile
	A550 × arg-10A	7	16	Large and many	VII	Good fertile
mat	$C_0$65 × I eu-3A	8	17	Large and some	I	Fertile
	$C_0$65 × arg-5A	8	17	Large and few	II	Less Fertile
	$C_0$65 × trp-1A	10	25	Small and 7-8	III	Less Fertile
	$C_0$65 × trp-4A	9	18	Small and some	IV	Fertile
	$C_0$65 × lysi-1A	10	25	Medium and few	V	Less Fertile
	$C_0$65 × trp-2A	7	15	Large and some	VI	Fertile
	$C_0$65 × arg-10A	8	21	Small and some	VII	Fertile

(contd...)

Table – 38 (contd...)

Name of the mutants	Name of the cross	Perithecia formed in days	Days taken for spore shedding	Size and number of perithecia	Linkage group of the marker	Fertility
buff	$P_2$1015 × I eu-3A	7	15	Large and many	I	Good Fertile
	$P_2$1015 × arg-5A	7	15	Small and many	II	Good Fertile
	$P_2$1015 × trp-1A	7	14	Large and many	III	Good Fertile
	$P_2$1015 × trp-4A	8	16	Medium and some	IV	Fertile
	$P_2$1015 × lysi-1A	10	25	Small and 10-12	V	Less Fertile
	$P_2$1015 × trp-2A	8	21	Medium and some	VI	Fertile
	$P_2$1015 × arg-10A	7	14	Medium and many	VII	Good fertile
fluffy band	$C_1$149 × leu-3A	10	23	Medium and few	I	Less Fertile
	$C_1$149 × arg-5A	10	25	Medium and 15-16	II	Less Fertile
	$C_1$149 × trp-1A	8	18	Large and some	III	Fertile
	$C_1$149 × trp-4A	8	17	Large and many	IV	Fertile
	$C_1$149 × lysi-1A	8	21	Medium and 5-6	V	Less Fertile
	$C_1$149 × trp-2A	9	18	Medium and some	VI	Fertile
	$C_1$149 × arg-10A	9	21	Small and some	VII	Fertile

5.7 : STUDY OF LINKAGE OF SOME SELECTED MUTANTS WITH THE MARKERS OF DIFFERENT LINKAGE GROUPS.

For study of linkage the spores from the cross were spread on supplemented SM plates. Then they were incubated at 58^0C for 50 minutes. The wild type spores grew after 12 hours, but the spores of mutants germinates only. Parallel lines were drawn on the lower lid of the plate and the germinating and growing spores were counted. The germinating and growing spores were isolated randomly and the spores were inoculated into small tubes containing VM supplemented with necessary amino-acid. The culture were than incubated at 25^0C. After 4 or 5 days, the isolates were tested on SM and supplemented SM plates. Thus the biochemical mutants of the progenies were identified.

5.7.1 : LINKAGE OF ROPY ($A_0$307).

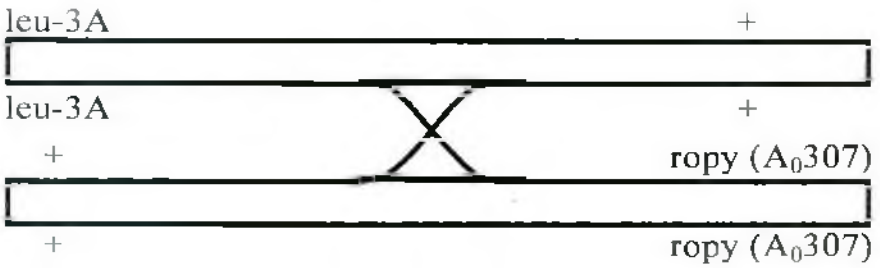
Table – 39

Linkage of ropy ($A_0$307)

Mutant used	Marker used	Linkage group	Cross	Progenies	% of each progeny	Inference on linkage
ropy ($A_0$307)	leu-3 (R156) A	I	roy X leu- 3A	a. lew-3A=26 b. ropy= 30 c. ro+leu-3A=16 d. wild = 14	a. 30.23% b. 34.88% c. 18.60% d. 16.27%	Linked with leu- 3A of linkage group I
ropy ($A_0$307)	trp-1 (10575) A	III	roy X trp-1A	a. trp-1A=25 b. ropy= 30 c. ro+trp-1A=31 d. wild = 39	a. 20% b. 24% c. 24.8% d. 31.2%	Not linked with trp-1A of linkage group III
ropy ($A_0$307)	trp-4 (Y2198) A	IV	roy X trp-4A	a. trp-4A=30 b. ropy= 35 c. ro+trp=29 d. wild = 28	a. 24.59% b. 28.68% c. 23.77% d. 22.95%	Not linked with trp-4A of linkage group IV
ropy ($A_0$307)	trp-2 (75001) A	VI	roy X trp-2A	a. trp-2A=20 b. Ropy= 30 c.Ro+trp-2A=25 d. wild = 30	a. 19.04% b. 28.57% c. 23.80% d. 28.57%	Not linked with try-1A of linkage group VI
ropy ($A_0$307)	arg-10 (B317) A	VII	roy X arg-10A	a. arg-10A=32 b. Ropy= 27 c.Ro+arg-10A=30 d. wild = 31	a. 26.67% b. 22.5% c. 25% d. 25.83%	Not linked with arg-10A of linkage group VII

5.7.2: DETERMINATION OF GENETIC MAP OF ROPY (A_0307)

Crossing name: ropy (A_0307) × leu–3 (R156)A



$$\text{Distance} = \frac{\text{Total wild recombinant}}{\text{Total progenies}} \times 100$$

$$= \frac{16+14}{86} \times 100$$

$$= 34.88 \text{ centimorgan}$$

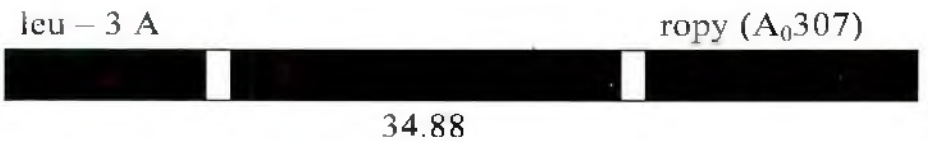


Fig: 17 Genetic map of ropy. (A_0307)

5.7.3 : LINKAGE OF CONIDIAL BAND ($P_0$729).

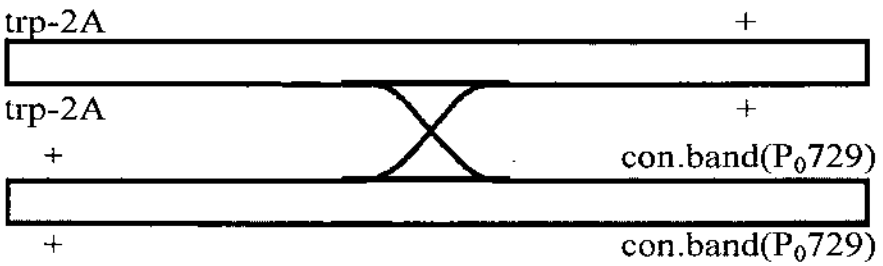
Table – 40

Linkage of con. band ($P_0$729)

Mutant used	Marker used	Linkage group	Cross	Progenics	% of each progeny	Inference on linkage
Conidi - al band ($P_0$729)	leu-3 (R156) A	I	Conidial band x I eu- 3A	a. leu-3A=20 b.con. band= 30 c. con. band + leu-3A=25 d. wild = 23	a. 20.40% b. 30.61% c. 25.51% d. 23.46%	Not linked with leu-3A of linkage group I
Conidi - al band ($P_0$729)	tr p-1 (10575) A	III	conidial band X tr p-1A	a. trp-1A=22 b. con. band= 32 c. con. band+trp-1A=22 d. wild = 24	a.22% b. 32% c. 22% d. 24%	Not linked with trp-1A of linkage group III
Conidi - al band ($P_0$729)	tr p-4 (Y2198) A	IV	conidial band X tr p-4A	a. trp-4A=30 b. con. band= 24 c. con. band+trp-4A=24 d. wild = 19	a. 30.92% b. 24.74% c. 24.74% d. 19.58%	Not linked with trp-4A of linkage group IV
Conidi - al band ($P_0$729)	tr p-2 (75001) A	VI	conidial band X tr p-2A	a. trp-2A=25 b. con. band= 35 c. con. band+trp-2A=14 d. wild = 11	a. 29.41% b. 41.17% c. 16.47% d. 12.94%	Linked with try-2A of linkage group VI
Conidi - al band ($P_0$729)	arg-10 (B317) A	VII	conidial band X arg-10A	a. arg10A=30 b. con. band= 35 c. con. band+arg-10A=30 d. wild = 25	a. 24% b. 29.16% c. 24% d. 20.83%	Not linked with try-10A of linkage group VII

5.7.4: DETERMINATION OF GENETIC MAP OF CONIDIAL BAND (($P_0$729).

Crossing Name : con. band ($P_0$729) × trp – 2 (75001) A



$$\text{Distance} = \frac{\text{Total wild recombinant}}{\text{Total progenies}} \times 100$$

$$= \frac{14+11}{85} \times 100$$

$$= 29.41 \text{ centimorgan}$$

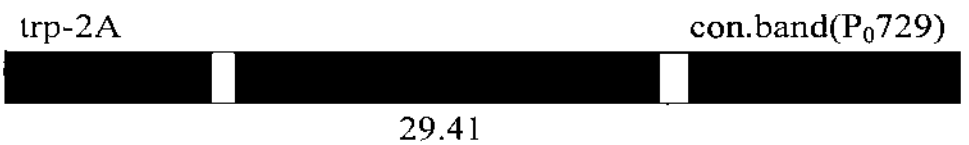


Fig. 18 : Genetic map of con. band ($P_0$729)

5.7.5 : LINKAGE OF CHECKED (A_0380)

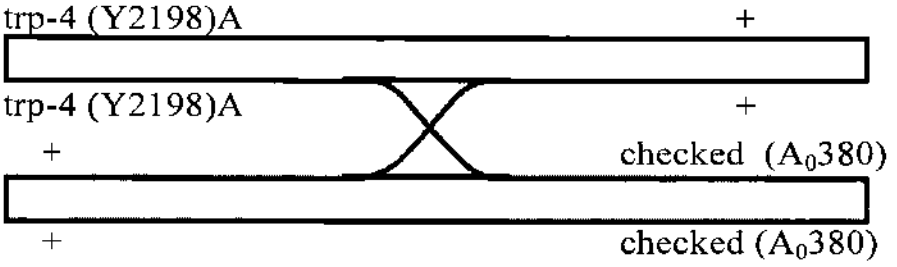
Table – 41

Linkage of checked (A_0380)

Mutant used	Marker used	Linkage group	Cross	Progenies	% of each progeny	Inference on linkage
checked (A_0380)	leu-3 (R156) A	I	checked (A_0380)×Leu- 3A	a. leu-3A=26 b.ch. = 20 c. ch+ leu-3A=18 d. wild = 20s	a. 30.95% b. 23.80% c. 21.42% d. 23.806%	Not linked with leu-3A of linkage group I
checked (A_0380)	trp-1 (10575) A	III	checked (A_0380) X trp-1A	a. trp-1A=24 b. ch= 21 c. ch+trp-1A=19 d. wild = 23	a.27.58% b. 24.13% c. 21.83% d. 26.43%	Not linked with trp-1A of linkage group III
checked (A_0380)	trp-4 (Y2198) A	IV	checked (A_0380) X trp-4A	a. trp-4A=35 b. ch= 36 c. ch+trp-4A=19 d. wild = 15	a. 33.33% b. 34.28% c. 18.09% d. 14.28%	Linked with trp-4A of linkage group IV
checked (A_0380)	trp-2 (75001) A	VI	checked (A_0380) X trp-2A	a. trp-2A=15 b. ch= 27 c. ch+trp-2A=13 d. wild = 30	a. 17.64% b. 31.76% c. 15.29% d. 35.29%	Not Linked with try-2A of linkage group VI
checked (A_0380)	arg-10 (B317) A	VII	checked (A_0380) X arg-10A	a. arg-10A=13 b. ch= 18 c.ch+arg-10A=15 d. wild = 30	a. 17.10% b. 23.68% c. 19.73% d. 39.47%	Not linked with try-10A of linkage group VII

5.7.6: DETERMINATION OF GENETIC MAP OF CHECKED ($A_0$380)

Crossing name : checked ($A_0$380) × trp-4 (Y2198) A



$$\text{Distance} = \frac{\text{Total wild recombinant}}{\text{Total progenies}} \times 100$$

$$= \frac{19+15}{105} \times 100$$

$$= 32.38 \text{ centimorgan}$$

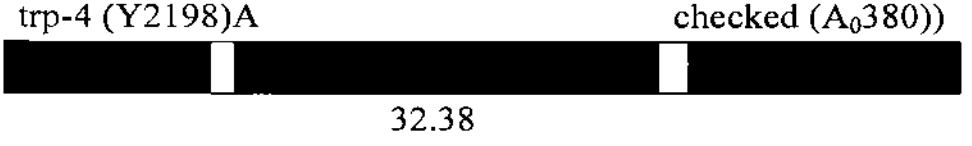


Fig : 19 Genetic map of checked ($A_0$380)

5.7.7 : LINKAGE OF FLUFFY (P_2945)

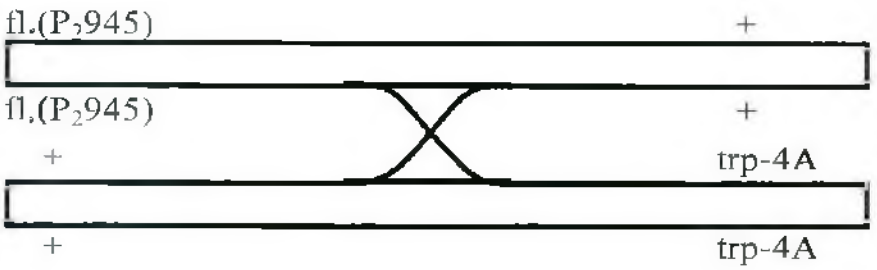
Table – 42

Linkage of fluffy ((P_2945))

Mutant used	Marker used	Linkage group	Cross	Progenies	% of each progeny	Inference on linkage
fluffy (P_2945)	leu-3 (R156) A	I	fluffy× I eu– 3A	a. lew-3A=30 b. fluffy = 32 c. fl+leu-3A=30 d. wild = 33	a. 24% b. 25.6% c. 24% d. 26.4%	Not linked with leu- 3A of linkage group I
fluffy (P_2945)	tr p-1 (10575) A	III	fluffy× trp-1A	a. trp-1A=25 b. fluffy = 20 c. fl+trp-1A=23 d. wild = 22	a. 27.7% b. 22.2% c. 25.5% d. 24.4%	Not linked with trp-1A of linkage group III
fluffy (P_2945)	tr p-4 (2198) A	IV	fluffy× trp-4A	a. trp-4A=30 b. fluffy = 35 c. fl+trp- 4A=15 d. wild = 16	a. 31.2% b. 36.4% c. 15.6% d. 16.6%	linked with trp-4a of linkage group IV
fluffy (P_2945)	tr p-2 (75001) A	VI	fluffy× trp-2A	a. tryp-2A=15 b. fluffy = 10 c.fl+tryp-2A=30 d. wild = 38	a. 16.1% b. 10.7% c. 32.2% d. 40.8%	Not linked with trp-2A of linkage group VI
fluffy (P_2945)	arg-10 (B317) A	VII	fluffy× arg-10A	a. arg-10A=18 b fluffy = 13 c.fl+arg-10A=30 d. wild = 29	a. 207% b. 14.4% c. 33.3% d. 32.2%	Not linked with arg-10A of linkage group VII

5.7.8: DETERMINATION OF GENETIC MAP OF FLUFFY($P_2$945)

Crossing name : fluffy ($P_2$945) × trp- 4 (Y2198) A



$$\text{Distance} = \frac{\text{Total wild recombinant}}{\text{Total progenies}} \times 100$$

$$= \frac{15+156}{96} \times 100$$

$$= 32.29 \text{ centimorgan}$$

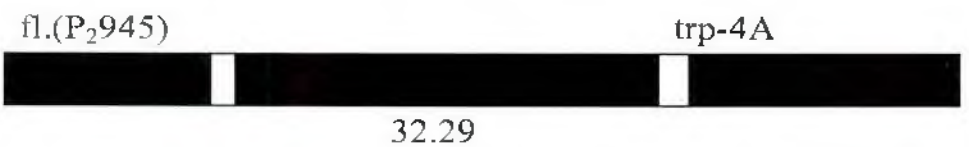


Fig : 20 Genetic map of fluffy ($P_2$945)

5.7.9: LINKAGE OF VIGOROUS ($A_1$550)

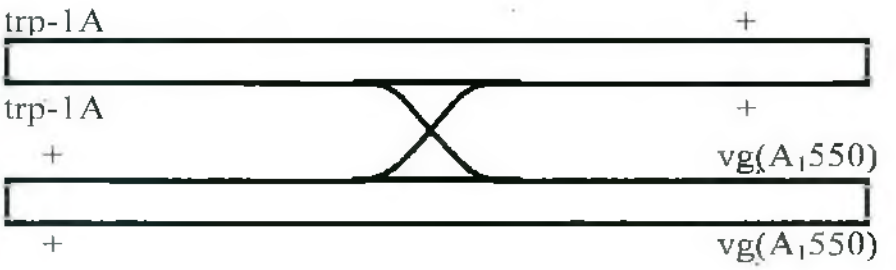
Table – 43

Linkage of Vigorous ($A_1$550)

Mutant used	Marker used	Linkage group	Cross	Progenies	% of each progeny	Inference on linkage
vigorous ($A_1$550)	leu-3 (R156) A	I	vigorous × I eu– 3A	a. leu-3A=28 b. vig = 20 c.vig+leu-3A=23 d. wild = 29	a. 28% b. 20% c. 23% d. 29%	Not Linked with leu- 3A of linkage group I
vigorous ($A_1$550)	trp-1 (10575) A	III	vigorous × trp-1A	a. trp-1A=38 b. vig = 40 c.vig+trp-1A=10 d. wild = 8	a. 39.58% b. 41.67% c. 10.41% d. 8.33%	Linked with trp-1A of linkage group III
vigorous ($A_1$550)	tr p-4 (Y2198) A	IV	vigorous × trp-4A	a. trp-4A=22 b. vig = 25 c.vig+trp- 4A=30 d. wild = 12	a. 24.71% b. 28.08% c. 33.70% d. 13.48%	Not linked with trp-4a of linkage group IV
vigorous ($A_1$550))	trp-2 (75001) A	VI	vigorous × trp-2A	a. trp-2A=19 b. vig = 35 c.vig+trp-2A=22 d. wild = 24	a. 19% b. 35% c. 22% d. 248%	Not linked with trp-2A of linkage group VI
vigorous ($A_1$550)	arg-10 (B317) A	VII	vigorous × arg-10A	a. arg-10A=25 b vig = 20 c.vig+arg-10A=30 d. wild = 15	a. 27.78% b. 22.22% c. 33.33% d. 16.67%	Not linked with arg-10A of linkage group VII

5.9.10 : Determination of genetic map of vigorous ($A_1$550)

Crossing name: vigorous ($A_1$550) × trp-1 (10575) A



$$\text{Distance} = \frac{\text{Total wild recombinant}}{\text{Total progenies}} \times 100$$

$$= \frac{10+8}{96} \times 100$$

$$= 18.75 \text{ centimorgan}$$

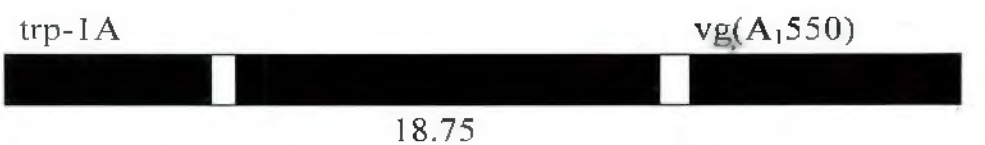


Fig : 21 Genetic map of vigorous ($A_1$550)

DISCUSSION

DISCUSSION

Stadler *et. al* (1993) reported that spontaneous mutation in *Neurospora crassa* is very rare and infrequent in nature. The author checked if there was any spontaneous mutation in *Neurospora crassa.* In this investigation conidia from fresh culture of Ema (5297) were spread on SM plates. Distinctly grown single conidial colonies were isolated individually in VM tubes. After 4-5 days when sufficient conidia were formed, individual cultures were analysed. None of the isolates were found to show morphological variations and all of them resembled the parental stock. It seems that there was no spontaneous mutation in *Neurospora crassa* in Laboratory stock which the author used for the induction of mutation. The previous research workers of Microbial Genetics Laboratory, Department of Botany, University of Dhaka also did not obtain any spontaneous mutations (Mozmader *et al* 1997).

The types of mutants obtained following the treatments with leaf extract of *Adhatoda zeylanica* Nees, leaf extract of *Centella asiatica* (Linn.) Urban and fruit extract of *Phyllanthus emblica* (Linn.). The variants with changed morphology were classified into 12 groups- ropy, checked, vigorous, plug, fluffy band, mat, albino, conidial band, cauliflower, buff, crescent and fluffy. The results are slightly different from that found earlier with formaldehyde vapour (Mozmader

et al 1995), formaldehyde solution (Mozmader *et al* 1997) and phenol solution (Mozmader *et al* 1986).

This discrepancies could be due to the difference in mutagenic specificity of leaf extract of *Adhatoda zeylanica* Nees, leaf extract of *Centella asiatica* (Linn.) Urban and fruit extract of *Phyllanthus emblica* (Linn.).

Fresh conidia of the selected representatives [ropy (A_o307), checked (A_o380), vigorous (A_1 550), plug (C_o 20), fluffy band ($C_1$149), mat (C_o 65), conidial band (P_0 729), albino (P_2 919), cauliflower ($A_1$415), buff ($P_2$1015), crescent ($A_1$515) and fluffy ($P_2$945)] were inoculated in VM plates for conidial germination and observations were recorded upto 7 hours.

Table 32 show that there was no indication of germination of conidia or mycelial growth upto 2 hours in the wild types Ema (5297) or any of the mutants . After 4 hours, growth was initiated in wild type Ema (5297) as well as in mutants namely con. band (P_o729), vigorous ($A_1$550), fluffy band ($C_1$149), fluffy ($P_2$945) and plug (C_o20) germinated after 3 hours.

Fresh conidia were inoculated in VM plates for determination of radial growth of the wild type and some selected mutants namely plug ($C_0$20), vigorous ($A_1$550), fluffy band ($C_1$149), con. band (P_0 729), ropy ($A_0$307), checked (A_o380), albino ($P_2$919), mat (C_0 65), cr.

($A_1$515), clf. ($A_1$415), fluffy ($P_2$945) and buff ($P_2$1015). Observation after 6 hours upto 48 hours were recorded (Table 33).

After 36 hours wild type Ema (5297) grew upto 3.05 cm where as mutants plug (C_o20) grew upto 4.15 cm and mat (C_o 65) grew upto 1.85 cm.

Fresh conidia were also inoculated in race tube (Ryan *et al* 1943) for the study of linear growth of the mycelia of the mutants and observation were taken after 6 hours upto 96 hours (Table 34) when linear growth were undertaken interesting results were found as shown in Table 34. The mutants differed in their linear growth from the linear growth found with Ema 25.75 cm. The growth of plug (C_o20) was significantly higher 33.95 cm and the growth of mat (C_o65) was lower 6.36cm.

Fresh conidia were inoculated further in liquid VM media in conical flasks for obtaining biomass of the wild type Ema(5297) and some selected mutants ropy (A_o307), albino ($P_2$919), con. band (P_o729), checked (A_o 380), vigorous ($A_1$550), plug (C_o20) fluffy band (C_1 149), mat (C_o65), cauliflower ($A_1$415), buff ($P_2$1015), fluffy ($P_2$945) and crescent ($A_1$515). Mycelia were harvested by filtering after 72 hours, washed with distilled water and dried in the oven at 58^0C and weighed (Table 35).

After 72 hours wild type Ema (5297) gave a mass of 0.1154g. The maximum mass (0.13725g.) was obtained in case of plug (C_o20) and the lowest (0.08445) was obtained in case of albino (P_2919).

Mating type and fertility of selected leaf extract and fruit extract induced mutants were determined .The mutants did not show variations in their mating types but different mutants behaved differently. Majority of them were good fertile, some of them were fertile and some of them were less fertile (Table-36).

Selected mutants were crossed with wild type (Ema and Em A). Spores were isolated and classified into mutant and wild. Their segregation data were given in Table-37. It was observed that the wild type and mutant progenies were segregating in 1:1 ratio. This finding suggests that the morphological mutants might be the results of changes in the nuclear gene.

The selected mutants were also crossed with the representative of different linkage groups. From table- 39, 40, 41, 42 and 43 it was found that mutant ropy (A_o307) was found to be linked with leu-3 (R-156) A of linkage group I and gave a distance 34.88 centimorgan. Mutant con. band (P_o729) was found to be linked with trp-2 (75001)A of linkage group VI and gave a distance 29.41centimorgan.Mutant checked (A_o 380) was found to be linked with trp-4 (Y2198)A of linkage group IV and gave a distance of 32.38 centimorgan . Mutant

fluffy ($P_2$945) was found to be linked with trp-4 (Y2198)A of linkage group IV and gave a distance 32.29 centimorgan. Mutant vigorous ($A_1$550) was found to be linked with trp-1(10575)A of linkage group III and gave a distance 18.75 centimorgan.

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