INDUCTION OF MORPHOLOGICAL MUTANTS IN *Neurospora crassa* AND IT S GENETICAL INVESTIGATION

A THESIS
SUBMITTED IN PARTIAL FULFILMENT FOR
THE DEGREE OF MASTER OF PHILOSOPHY
TO THE DEPARTMENT OF BOTANY
UNIVERSITY OF DHAKA

By
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Dedicated to my
beloved teacher
Dr. Tahsina Rahim
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CERTIFICATE

I hereby crtify that the research work embodying the results reported in the thesis entitled **"Induction of morphological mutants in *Neurospora crassa* and its genetical investigation",** submitted by Hasna Israt Naznine Banu bearing registration no. 226, session 2002-2003 for submission in the form of thesis has been carried out under my guidance for the degree of Master of Philosophy in Botany, University of Dhaka.

Tahsina Rahim
10.4.08

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Professor and supervisor
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DECLARATION

I do hereby declare that the work submitted as a thesis entitled, **"Induction of morphological mutants in *Neurospora crassa* and its genetical investigation"** to the department of Botany, University of Dhaka for the degree of Master of Philosophy by the result of my own investigation and carried out under the supervision of Dr. Tahsina Rahim, Professor of Botany, University of Dhaka. The research work has not previously been submitted for any degree.

10.09.03

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LIST OF ABREVIATION

Em	= Emerson stock
Em a	= Emerson stock mating type 'a'
Em A	= 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	= Westergard's crossing medium
cm	= centimeter
g	= gram
ml	= mililiter
%	= percent
leu-3	= leucine-3 auxotroph
ad-1	= adenine-1 auxotroph
arg-10	= argenine-10 auxotroph
leu	= leucine
arg	= argenine
his	= histidine
lys	= lysine
trp	= triptophane
UV	= ultraviolet
fl	= *fluffy*
con. band	= *conidial band*
vg	= *vigorous*
ro	= *ropy*
con.lump	= *conidial lump*
AA	= Anthranilic acid
Anth. Syn	= Anthranilic synthetase
C	= Conidia form

ABSTRACT

It was found that the concentration of leaf extract of *Datura metel* Linn., *Eclipta prostrata* Linn *and* rhizome extract of *Asparagus racemosus* Linn. were controlled radial growth of willd type *Neurospora crassa* (Ema). The non sterilized concentration was more effective than sterilized concentration. Nonsterilized concentration was the most effective and the sterilized concentration was least effective.. Non sterilized concentration were chosen for treating the conidia of Em'a' for induction of mutation and nine group of mutants were found. These are *ropy, conidial band, vigorous, conidial lump,plug,albino, fluffy and buff.*

It was also found that UV light of 254nm wavelength was very effective for artificial induction of mutation and Five groups of morphological mutants *ropy, albino, fluffy, check and vigorous.* were obtained.

The mycelia of *ropy* form a rope like structure and produces pink colour conidia. A profused conidial and mycelial growth race the plug of *vigorous* which is deep pink in colour. *conidial lump* is a pink coloured lumped structure. A densed pink conidial growth formed outside the tube and conidia forme around the *plug. albino* contains mycelia very scanty in number and growth is less and conidia completely colourless. In *fluffy,* mycelia is cottony and compactly arranged but in *buff* mycelia and conidia are coloured.

A good number of morphological mutants were induced with leaf extract of *Datura metel* Linn., leaf extract of *Eclipta prostrata* Linn., rhizome extract of *Asparagus racemosus* Linn.and UV irradiation were selected for detailed study and analysis.

Comperative study of Em'a' and the selected morphological mutants were carried out. These mutants showed clear variation and some were very much different from the wild stock. These variations were confirmed strikingly fitted result obtained from the dry weight of the mycelia, initiation of conidial germination, linear growth and radial growth.

The mutants were crossed with Em'a' and Em'A' separately and their segregating ratio were studied. Mating type of some extracts was also checked. The progenies showed about 1:1 ratio in their morphology and mating type mutant. So it was concluded that the mutant characters have resulted due to mutation in their nuclear gene.

Linkage study of nine selected mutants *ropy, fluffy, vigorous, conidial band, albino, buff, conidial lump, plug* were determined distances of the mutants with the respective markers were calculated and given below:

Mutant vigorous was found to be linked with leu-3(R156)A of linkage group I and gave a distance of 31.13 centimorgan. Mutant fluffy and conidial band were found to be linked were trp-4A of linkage group IV and gave a distance of 29.17 and 19.17 centimorgane respectively. Mutant Ropy was found to be linked with Leu-3A of linkage group I and gave distance of 34.73 centimorgan and Mutant buff was found to be linked with arg-10A of linkage group VII and gave a distance of 31 centimorgan.

CHAPTER1 - 1

INTRODUCTION

INTRODUCTION

Neurospora crassa is the most exciting material for genetic study. Gentic work with this has effectively began 56 years ago. Their short life cycle and capacity to grow in minimal growth requiring substances help the scientists of this branch to perform extensive work within short time, which is one of the most essential factors of genetical study.

Neurospora crassa, the well known pink bread mold, which is also called plant *Drosophila* grows in tropical and sub tropical regions.It is a filamentus fungus that belongs to the class- *Ascomycetes* sub class- *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 born on the same hermaphroditic mycelium. The male organ is the protoperithecium, which cinsists of a coiled cell, the ascogonium, extending into are respective hyphae enclosed by a small knot of hyphae. The male function of the fertilization of the ascogonium by strain of the order mating type can be carried out (most commonly) by a conidium or by a suitably placed hyphae.

Neurospora crassa has proved to be an important genetic material for its profuse vegetative growth and sexual reproduction in synthetic media. It is haploid, heterothellic, octoporous having linear arrangement of its eight ascopores in an ascus and as a result and it can be used for any classical as well as molecular investigations. The idea of taking this particular species of fungus is that it has short life

cycle and hence suitable for genetical studies. *Neurospora crassa* is octoporous, hermaphoroditic, heterothallic and as a result there are two mating types called 'A' and 'a'.

Neurospora crassa is non pathogenic and can be easily grown on a suitable mixture of material salt in water plus a carbon source (sucrose) and the vitamin biotin (Beadle and Tatum, 1941; Annonymous, 1966). Both growth on race tube (Ryan *et al* 1943) and dry weight of mycelia (Beadle and Tatum, 1941) are useful for accurate measurement of growth, the former measuring rate and the latter terminal growth mass.

This fungus has many advantages in the study of what genes can do. It's genes can conveniently be changed artificially and the part they play in the chemical alteration and metabolism of cells can be analyzed with considerable precision (George,1984). It can be multiplied a million fold in a few days without any genetic change (Pandit and Maheswari, 1993).

Genetic analysis began by Morgan in 1909 and by the subsequent studies arose the concept of gene as a unit of recombination (A.H. Sturtevant, 1913) when in 1927 at the 5th International congress of genetics in Berlin, Muller from United States first reported production of mutation in *Drosophila* by X- rays, he opened a new chapter in the history of genetics (C. Auerbach, 1962).

As the ascospores mature they develop thick black cell walls which make them highly resistant and dormant. The ripe ascus eventually ruptures at its tip and the eight ellipsoidal spores are discharged through the neck of the perithecium. Germination of the ascopores is induced by heat shock (30-45 minutes at 60°C or 50 minutes at 58°C) in different laboratories(Fincham 1983).

The study of genetics of *Neurospora crassa* is of great value for a variety reasons. It has provided geneticists with novel genetic

systems and with the opportunity of studying particular problems with greater ease or precision that is possible in other organism. And as a result the genetics of *Neurospora crassa* have been studied fairly intensively during the recent years (Aranson *et al* 1994; Akinson *et al* 1995; Bruchez *et al* 1992; Kappor *et al* 1995; McDonald *et al* 1995; Maoisel *et al* 1995; Crawford *et al* 1992; 1993; Fossa *et al* 1995; Kuldau *et al* 1993; Pall *et al* 1993; Yassin *et al* 1992).

Neurospora crassa is heterotrophic for carbon compounds. It is also hetrotophic for vitamins such as thiamin, biotin or pyridoxine. Its nutritional requirements can be met in culture by supplying simple sugar, such as glucose or sucrose, mineral salt including a source of inorganic nitrogen and water. It can often be grown also in plate cultures on simple defined media. The change in phenotype could be defined much more precisely in chemical terms.

The ability of the fungus to produce large number of identical predominantly haploid, uninucleate conidia enables such units to be used for the investigation of spontaneous (Stadler et al 1993, Watters et al 1995)and natural mutations since conidia can be readily exposed to a wide variety of mutagens they are of particular value in mutation studies.

Much of the preliminary genetic work on *Neurospora crassa* had already been published by Dodge, B.O.(1932, 35 and 42) and Lindegren, C.C.(1932 and 19330. then the break through came in the early 1940's when Beadle and Tatum (1941), began their work on the fungus *Neurospora.* The concept of one to one relationship between zenes and enzymes or between mutations and block or between gene to polypeptide in metabolic pathway was almost as much of a rediscovery as was the rediscovery of Mendel's work in 1900. Both biochemists and geneticists now bad advanced to the point where

effective use could be made on the concept, and the work quickly grew and expanded in many fruitful ways.

Initiated by the investigation of Beadle and Tatum (1941) it is now known that DNA determines the structure of protein. DNA controls the biochemical activities of cell by determining the structure of specific enzymes and moreover specific molecules of DNA determine the specific amino acid sequences of specific protein chain.

What are the mutagene 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 Tatum1945; Bonner *et al* 1994b and 1995; Santosa *et al* 1992; Stephens *et al* 1993; Velangi *et al* 1994 and Yu *et al* 1993).

The term of mutation was first used by Wagon in 1869. Hugo De Vries (1901), the famous Dutch Botanist, brought into general use of the world "mutation". Genetic analysis began by Morgan in 1909 and by the studies arose the concept of gene as a unit of recombination(A.H. Sturtevent 1913) when 1927 at the 5^{th} international congress of Genetics in Berlin, H.J.Muller from United States first reported the production of mutation in *Drosophila* by X-rays, he opened a new chapter in the history of genetics.

Mutagenes used by the geneticists may be of two kinds; those are physical and chemical. Physical mutagens include X-ray, UV-ray etc. In the study of genetics, UV-light of the wavelength 254 nm is the most commonly used mutagen.

Ultra-violet light seems to induce true gene mutations as well as loses reversion of the wild-type gene and perhaps also mutation to

the other alleles. Ultra-violet light, unlike X-rays does not induce gross structural changes in the chromosomes (Catcheside 1951; the genetics of microorganisms). The effect of ultra-violet photon is localized; it is limited to one atom, UV light has very weak penetrating power. It is especially effective as a mutagenic agent in micro organism, which because of their small sizes; present their genetic material almost directory to any UV light that impinges on their surface. The maximum absorption of UV by DNA is at a wavelength of 254nm.Maximum mutagenecity also occurs at 254 nm .UV radiation of 254 nm was used for induction of mutation.

In research on microorganism UV radiation serves the purpose more effectively. Most of the mutants for the study of gene action in micro organism were produced by exposure of cell to UV light (Auerbach C; The science of Genetics). In the genetic research laboratory of Dhaka University,Ahmad and Mazmader in 1965 irradiation the conidia of *Neurospora crassa* strain Emersion'a'(5297) with UV light and isolated a number of biochemical mutants along with the morphological mutants which are studied by Begum A (1966)

The production of mutation by chemical treatment of cell was first reported by Steingberg in 1939 (A.M. Winchester,Genetics: a survey of the principles of heredity). Auerhach and Robson discovered that mustard gas is a powerful mutagenic agent (Edgar, Alterberg, Genetics).Study of Kolmarks and Westergaard have shown that Bromethyl methane sulphonate,when used on *Neurospora crassa* cause mutations of the gene ad- to ad+, but causes practically on mutation of inso- to inso + (A.M. Winchester). Admirable accounts both of genetics and much of biochemical work is given by Catcheside (1951) and Horowitz (1950) (J.B.S.Haldane . The Biochemistry of Genetics). Perhaps the most extensive and systemic surveys of the action chemical mutagens on fungi have been made by Westergaard on *Neurospora crassa* (J.R.S. Fincham and P.R.Day).

In the microbial genetics Labotatory of Dhaka University Mustafa,H. (1995) Hossan, F. (1995) and Sultana Rajia (1996) induced mutation in *Neurospora crassa* with formaldehyde solution and obtained morphlogical mutants of different groups. Mozmader and Tahaina (1995) induced mutation in *Neurospora crassa* withvapour of formaldehyde. Chowdhury (1974) induced morphological mutants in *Neurospora crassa* with vapour of formaldehyde. Dutto,K. (1982) induced morphological mutants in *Neurospora crassa* with form,aldehyde solution. Mozmader *et al* (1986) induced few morphological mutants by direct treatment with phenol solution.

The term linkage was first coined by Bateson and Pannett in 1909. Morgan's concept about the linkage developed the theory of linear arrangement of gene in the chromosomes which becomes helpful for linkage of chromosomes.

Linkage may be complete when the genes of one linkage group segregate tougheter or may be incomplete, when gene pairs in most linkage groups assort at least partially independently of each other (Morgan 1944).

The most distant genes on a chromosome may approach the 50% recombination level and they seem unlinked only when a third gene is discovered which shows a lower percentage of recombination with both distant genes, these two genes may acknowledge to be linked also, since linkage is reciprocal. Seven linkage groups have been identified in *Neurospora crassa,* in which haploid chromosome number in seven. Linkage study of the different mutant has one of the greatest significance for the living organisms, That it reduces the possibility of variability in gamates, unless crossing over occurs (Verma *et al* 1983) The study of linkage and recombination is basic

to genetic analysis and has enabled us to plot genetic map of chromosomes.

Linkage was first demonstrated in *Neurospora crassa* by Lindegren(1933). Subsequently he published linkage maps of linkage group I (Lindegren, 193) and II
(Lindegren and Lindegren 1939).

Therefore a plan was to determine

(i) If leaf extract of *Datura matel* Linn., *Eclipta prostrate* Linn, Rhizome extract of *Asparagus racemosus* Linn.and UV can produce new morphological mutants.
(ii) Whether the obtained mutants resulted due to the mutation in nuclear gene by crossing them with Em'a' and Em'A' separately and by studying their segregation.
(iii) The linkage of some selected mutants by crossing them with marker representative of all the seven linkage groups and prepare genetic map of some selected mutants.

CHAPTER-2

EXPERIMENTAL MATERIALS

EXPERIMENTAL MATERIALS

The experimental material was *Neurospora crassa,* a fungus belonging to the class ascomycetes. This material was kindly supplied by Dr.T.I.M.A.Mozmader, Professor, Department of Botany, University of Dhaka.

Neurospora crassa exist as a soil saprophyte. It can grow on sugarcane bagesse, carbonized vegetation or in bakeries. It is an important pest on bread and flour, leading to its characterization as the 'Pink Bread Mold'. This fungus possesses interwined segmented. Branched hyphal filamented some 5 μ or less in diameter. Each hyphal segment contains a haploid nucleus with seven chromosomes. It reproduces both asexually and sexually. Asexual spores are called conidia and sexual spores are called ascospores. Sexual fruiting bodies i.e perithecia are produced by the contact of hyphace of two opposite mating types known as Em 'A' and Em 'a' strains when grown on a suitable agar crossing media (Westergaard and Mitchell, 1947; Anynomous 1966) [Robert C. King, Hard book of genetics, vol-1, P-514] Conidia germinates readily in a few hours when transferred to a suitable culture media.

Culture Emerson 'a' (Em 'a') (5297) Emerson 'A' (Em 'A') (5296) and makers were used for the investigation. Em 'a' was used for treating conidia with mutagens and for comparing growth of mutants. Both Em 'a' and Em 'A' were used for checking mating types of mutants and extract 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 Centre, 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.

1. Wild type

1) Emerson 'A' (Em 'A')
2) Emerson 'a' (Em 'a')

2. STANDARD MARKERS

Name of the markers	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
ad-1	3254	A	VIR	VI
arg-10	B 317	A	VIIR	VII

3. MUTAGEN

A. 1)Physical Mutagen
2) UV Irradiation
UV Radiation of 254 nm.

B. Plant extract

a) Plant extract of *Datura metel* Linn.

Scientific name	:	*Datura metel* Linn.
Bengali name	:	Datura,Dhutura
Enlish name	:	Thorn apple
Family	:	Solanaceae
Useful part	:	Leaf

b) Leaf extract of *Eclipta prostrata* Linn.

Scientific name	:	*Eclipta prostrata* Linn.
Bengali name	:	Kessuti, Kesaraj.
Enlish name	:	Eclipta
Family	:	Asteraceae
Useful part	:	Leaf

c) Rhizome extracts *Asparagus racemosus* Linn.

Scientific name	:	*Asparagus racemosus* Linn.
Bengali name	:	Shatamuli
Enlish name	:	Asparagus
Family	:	Liliaceae
Useful part	:	Rhizome

4. Apparatus and glassware which were used for the present research works are as follows

1. Apparatus
2. Autoclave
3. Centrifuge
4. Refrigerator
5. Electric balance
6. Incubator
7. Inoculation chamber
8. pH meter
9. Shaker Gy Rotary
10. Water bath
11. Air pump
12. Ultra violate lamp

Glassware

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"×1.5"	Growth race tubes
7	Petri dishes	Pyrex	5" dia	Irradiation
8	Petridishes	Pyrex	3" dia	Media
9	Flasks(Eelnmyer)	Pyrex	100 ml	Media and water etc
10	Flasks(Eelnmyer)	Pyrex	250 ml	Media and water etc
11	Flasks(Eelnmyer)	Pyrex	500 ml	Media and water etc
12	Flasks(Eelnmyer)	Pyrex	1000 ml	Media and water etc

5. CHEMICALS USED FOR THE RESEARCH WORK

a) General

1) Agar(Bacto)
2) Ammonium nitrate(NH4 NO3)
3) Ammonium sulphate(NH4SO4)
4) Biotin (C10H16N2O3S)
5) Boric acid (H3BO4) Anhydride
6) Calcium chloride (CaCl2. 6H2O)
7) Chloroform (CHCl3)
8) Citric acid [C(OH)(COOH)(CH2OOH)2.H2O]
9) Copper sulphate (CuSO4.7H2O)
10) Ferous sulphate(FeSO4.7H2O)
11) Hydrocloric acid(HCl)
12) Magnesium chloride(MgCl2)
13) Magnesium sulphate(MgSO4.7H2O)
14) Manganese sulphate(MnSO4.7H2O)
15) Methanol (CH3OH)
16) Potassium nitrate (KNO3)
17) Potassium di-hydrogen phosphate(KH2PO4)
18) Sodium chloride(NaCl)
19) Sodium citrate (NaH2C6H5O7.2H2O)
20) Sodium molybdate (NaM0O4.2H2O)
21) Sorbose [OCH2 (CHOH)3]
22) Sucrose (Cl2H22O11)
23)Zinc sulphate(ZnSO4.7H2O)

b) Amino acid Supplement

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

c) Special reagent

1. 0.05M 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. Phenolphthaline

d) Stains

1. Cotton blue
2. Acridine orange

CHAPTER - 3

MEDIA

MEDIA

a) Solution needed for different medium
b) Trace element solution
c) Biotin solution
d) Vogel's Stock solution
e) Westergaard's stock solution
f) Amino acid supplement
g) Vogel's minimal (VM) medium
h) Composition of Vogel's minimal medium
i) Vogel's minimal liquid medium
j) Medium for linear growth
k) Medium for plating
l) Sorbose minimal (SM) medium
m) Medium used for crossing
n) Westergaard's crossing medium
o) Suyama's crossing medium
p) Sterilization

MEDIA

Solution needed for different media

Wild type strain of *Nurospora crassa* **can** be grown ona suitable mixture of mineral salts water plus a carbon source (sucrose, glucose) and the vitamin biotin (Bedle and Tatum 1945, anonymous 1966).

For culturing *Nurospora crassa* various stock solutions were needed for the preparation of media. To prepare a stock solution, the chemicals were added one after another ands 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

Biotin solution

Composition of biotin per 100 ml

1. Distilled water	100 ml
2. Biotin	10 mg

This solution was needed for Vogel's stock solution and Westergaard's stock solution.

Composition of trace element solution per 100 ml

1. Distilled water	95 ml
2. Citirc acid C (OH) (COOH) (CH_2COOH)	5 g
3. Zinc sulphate ($ZnSO_4$, $7H_2O$)	5 g
4. Ferrous sulphate ($FeS0_4$, $6H_2O$)	1 g
5. Copper sulphate ($CuSO_4$, $5H_2O$)	0.25 g
6. Manganese sulphate ($MnSO_4$, H_2O)	0.65 g
7. Boric acid anhydrous (H_3BO_4)	0.05 g
8. Sodium molybdate ($NaMoO_4$, $2H_2O$)	0.05 g
9. Chloroform ($CHCl_3$)	1 ml

This solution is needed for the preparation of Vogel's stock solution.

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 1000 ml 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)	125 g
4. Ammonium Nitrate (NH_4NO_3)	50 g
5. Magnesium Sulphate ($MgSO_4$, $7H_2O$)	5 g
6. Calcium Chloride (Cacl2, $6H_2O$)	3.75 g
7. Trace elements solution	2.5 ml
8. Biotin solution	1.25 ml
9. Chloroform ($CHCl_3$)	2.0 ml

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5. Magnesium Sulphate ($MgSO_4$, $7H_2O$)	5 g
6. Calcium Chloride ($Cacl_2$, $6H_2O$)	3.75 g
7. Trace elements solution	2.5 ml
8 Biotin solution	1.25 ml
9. Chloroform ($CHCl_3$)	2.0 ml

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

Westergaard's stock solution

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

Cmposition of Westergaard's stock solution per liter

1. Distilled water	1000 ml
2. Potassium Nitrate (KNO_3)	4 g
3. Potassium Dihydrogen Phosphate (KH_2PO_4)	4 g
4. Magnesium Sulphate ($MgSO_4$, $7H_2O$)	4.1 g
5. Calcium Chloride ($CaCl_2$, $6H_2O$)	0.8 g
6. Sodium Chloride (NaCl)	0.4 g
7. Biotin solution	15 drops

8. Chloroform ($CHCl_3$) 2 ml

STOCK SOLUTION OF AMINO-ACIDS

The Solution was required for the preparation of supplemented 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 one litre of the medium.

The stock solution was named after the amino acid used; for example,tryptophan solution,arginine solution.Composition of amino acid solution was as follows:

1. Distilled water 100 ml
2. Amino acids 100 ml.

Composition of Vogel's Minimal Medium (V.M) per liter for culturing

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

This medium is used for sub culturing and heterocaryon test but in case of plating 2% of agar was used and in case of liner growth 4% agar was used. 2% glucose was used in lieu of sucrose.100 mg of required amino acid was used for supplemented VM per liter.

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	960 ml
2. Vogel's stock solution	40 g
4. Sucrose	20 g

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

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

The medium was supplemented with amino-acids where necessary.

MEDIUM FOR PLATING

Sorbose Minimal Medium

Sorbose minimal medium was used for plating. It restricts growth so that colonices do not run into each other which could happen in VM. The rapid liner growth rate of *Neurospora crassa* was a serious draw back 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 (Tatum et al 1949). The importance of the ratio between other carbon sources and sorbose had been investigated (Tatum et al 1949,
Brockman and De serres, 1964) [Robert C. King, Handbook of Genetics, Vol. 1, Page 514]. For the reason supplemented sorbose minimal medium as well as non supplemented sorbose minimal medium were used for plating. This medium was used for minimal test of wild type and mutants.

Composition of sorbose minimal (SM) medium per liter

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

Sorbose was added after boiling all other constituents to avoid caramelization.

MEDIUM USED FOR CROSSING

WESTGAARD'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	250 ml
2. Distilled water	750 ml
3. Sucrose	20 g
4. Agar	20 g

SUYAMA'S CROSSING MEDIUM

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

Composition of Suyama's crossing Medium per liter

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

In the upper two cases, amino-acid was added where necessary. For example incase of tryptophan mutants, 40 mg. of tryptophan per 1000 ml 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 tube (6"). The medium remained in furrows of the folded filter paper strip when the tubes were slanted

STERILIZATION

All the media and essential instruments were sterilized in the autoclave 120°C under 15 Lb pressure for 20 minutes. The inoculation chamber, needle was burnt over the flame of a sprit lamp

CHAPTER - 4

METHODS

METHODS

Stock maintaining

The stock of the cultures of the mutants, markers wild types (EmA and Ema) were maintained in stock tubes containing 5 ml media 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 ± 4° C. The cultures were found to produce conidia within 4 to 5 days.

SUBCULTURING

Generally 2½-3" 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 medim was supplemented with 10 mg/100 ml media of necessary amino-acid for sub culturing biochemical mutants and the markers.

Subculturing was done in a sterilized inculation 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°C for 4-5 days for the growth and formation of conidia.

Vogel's Minimal Medium

Vogel's minimal medium (VM) (Vogel 1956) was used for culturing *Neurospora crassa* in the test tube and counting the spores on plate. For plating of 10 ml of medium was taken on the large test tubes (6") and 2-4 ml of medium was taken in a small test tube (2.5"-3") for maintaining cultures and growing spores. Amino acid supplements were used in culturing medium according to the specific type of mutants. The supplemented media were named after the name of the amino acid used e.g. leucine medium, adenine medium etc.

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 any spontaneous mutation in *Neurospora crassa.* Several steps were taken finding out the spontaneous mutation in *Neurospora crassa.*

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 test tube was shaken well to make a homogenous suspension of conidia.

Centrifugation

16 ml of the suspension was taken in two centrifuge tubes, 8ml in each and the two centrifuged 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.

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 well for mixing the water with conidia properly.

Plating

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

Incubation

The Petri plates were incubated at 25°C for 18-24 hours for formation of colony.

Isolation

After growth of conidial colonics in SM Petri plates, single conidial colonics were isolated in fresh and sterilized VM in 1000 tubes with isolating needle. The isolates were incubated at 25°C for proper growth.

Incubation

The isolates were incubated at a temperature of 25°C for 3-4 days for the proper growth of conidiation of the inoculates.

Observation

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

STUDIES ON MUTAGENIC EFFECT OF LEAF EXTRCT OF *Detura metel* Linn. ON THE GROWTH OF *Neurospora crassa*

Preparation of solution of concentration of *Detura metal* Linn.

At first 50 gm of fresh raw leaves of *Detura metal* Linn. were measured with the help of a balance. The leaves were washed with tap water 2-3 times and finally it was washed with distilled water and air dried.A paste was made with the help of a electric grinder. The paste was filtered 2-3 times with the help of sterilized thick cotton and finally it was filtered with the help of sterilized filter paper. The whole filtration was done in a inoculation chamber to prevent contamination. The concentration was divided as D_O, D_1, D_2, D_3 and D_4 with VM& 1ml distilled water, 1, 2, 4 drops and 1ml leaf extract respectively. Each concentration taken in duplicates and one set was sterilized in the autoclave at 121°C under 15 lb pressure for 20 minutes and other set was kept at normal temperature of the laboratory.

EFFECT OF NRORMAL (UNAUTOCLAVED) AND AUTOCLAVED LEAF EXTRACT OF *Detura metel* Linn. ON THE GROWTH OF *Neurospora crassa*

At first the Petri dishes were marked as 0, 1, 2,4drops and 1ml with the help of glass marker. 1,2,4drops and 1ml of normal and autoclaved D_1,D_2,$D_3$and D_4 concentration were taken in separate sterilized Petri dish with the help of sterilized pipette. 10 ml of molten VM plating medium was added in each Petri dish and the Petri dishes were shaken gently for thorough mixing of the extracts. As for control VM plating medium was taken in each sterilized Petri dish. When the Petri dish was cooled 4 days old Em'a' was inoculated at the centre of the Petri dishes inside the inoculation chamber to prevent contamination. Test for both control and the leaf extract concentration was done in duplicate.The Petri dishes were kept in the incubator at 25°C and observed after17, 23, 39 and 46 hours.

TREATING OF Em 'a' WITH EXTRACT OF LEAF OF *Datura metel* Linn.

1.Obtaining of fresh culture Em'a'

For obtaining of fresh culture Em'a' was sub cultured 4 times after an interval of 4 days. In each case culture of 5 days old were used for treating conidia.

2. Sterilization

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

3. Centrifugation

8 ml of the solution of *Daturine leaf extract* of the concentration D_1, D_3, D_4 and D_5 were taken into for centrifuge tubes. 1 loop of conidia of Em'a' was taken into each tube and was shaken for homogeneous solution. The solution was centrifuged with the help of a centrifuge machine for 15-20 minutes.

4. Filtration

The solution of the centrifuge tubes were poured out carefully. So the conidia could remain in the bottoms of the tubes. 8 ml of sterilized distilled water was taken into each tube and centrifuged for 3 minutes. The upper `liquid was poured out. The same procedure of pouring out upper liquid, adding water and centrifugation was repeated 2 times.

5. Preparation of suspension with treated conidia

8 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 drop of the suspension was taken in each sterilized Petri dish. the Petri dish was marked as D_1,D_2,D_4 and D_5 previously and the suspension was taken accordingly. 10 ml of molten SM at 47°C was added to each Petri dish. The temperature of the media did not exceed 47°C. The suspension and media were mixed well by shaking the Petri dish gently.

The whole experiment was done in the chamber to avoid contamination.

7. Incubation

The Petri plates were incubated at 25°C for 18-24 hours for formation of colony.

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

9. Incubation

The isolates were incubated at a temperature of 25°C for 3-4 days for the proper growth of conidiation of the inoculates.

10. Classification

The cultures were observed after 5 days and classified by comparing their characters with wild type Em'a'

STUDIES ON MUTAGENIC EFFECT OF LEAF EXTRCT OF *Eclipta prostrata* Linn. ON THE GROWTH OF *Neurospora crassa*

Preparation of solution of concentration of *Eclipta prostrata* Linn.

At first 50 gm of fresh raw leaves of. *Eclipta prostrata* Linn. was measured with the help of a balance. The leaves were washed with tap water 2-3 times and finally it was washed with distilled water and air dried.A paste was made with the help of an electric grinder. The paste was filtered 2-3 times with the help of sterilized thick cotton and finally it was filtered with the help of sterilized filter paper. The whole filtration was done in a inoculation chamber to prevent contamination. The concentration was divided as D_O, D_1, D_2, D_3 and D_4 with VM & 1ml distilled water 1, 2, 4 dorps and 1ml leaf extract respectively. Each concentration taken in duplicate and one set was sterilized in the autoclave at 121°C under 15 lb pressure for 20 minutes and other set was kept at normal temperature of the laboratory.

EFFECT OF NRORMAL (UNAUTOCLAVED) AND AUTOCLAVED LEAF EXTRACT OF *Eclipta prostrata* Linn. ON THE GROWTH OF *Neurospora crassa*

At first the Petri dishes were marked as 0, 1, 2,4drops and 1ml with the help of glass marker. 1,2,4 drops and 1ml of normal and autoclaved D_1,D_2,D_3 and D_4 concentration were taken in separate sterilized Petri dish with the help of sterilized pipette. 10 ml of molten VM plating medium was added in each Petri dish and the Petri dishes were shaken gently for thorough mixing of the extracts. As for control VM plating medium was taken in each sterilized Petri dish. When the Petri dishes was cooled 4 days old Em'a' was inoculated at the centre of the Petri dishes inside the inoculation chamber to prevent contamination. Test for both control and the leaf extract concentration was done in duplicate.

The Petri dishes were kept in the incubator at 25°C and observed after17, 23, 39 and 46 hours.

TREATING OF Em 'a' WITH EXTRACT OF LEAF OF *Eclipta prostrata* Linn.

1. Obtaining of fresh culture Em'a'

For obtaining of fresh culture Em'a' was sub cultured 4 times after an interval of 4 days. In each case culture of 5 days old were used for treating conidia.

2. Sterilization

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

3. Centrifugation

8 ml of the solution of *leaf extract* of *Eclipta prostrata* Less of the concentration D_1, D_3, D_4 and D_5 were taken into for centrifuge tubes. 1 loop of conidia of Em'a' was taken into each tube and was shaken for homogeneous solution. The solution was ce4ntrifuged with the help of a centrifuge machine for 15-20 minutes.

4. Filtration

The solution of the centrifuge tubes were poured out carefully. So the conidia could remain in the bottoms of the tubes. 8 ml of sterilized distilled water was taken into each tube and centrifuged for 3 minutes. The upper liquid was poured out. The same procedure of pouring out upper liquid, adding water and centrifugation was repeated 2 times.

5. Preparation of suspension with treated conidia

8 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 drop of the suspension was taken in each sterilized Petri dish. the Petri dish was marked as D_1,D_2,D_4 and D_5 previously and the suspension was taken accordingly. 10 ml of molten SM at 47°C was added to each Petri dish. The temperature of the media did not exceed 47°C . The suspension and media were mixed well by shaking the Petri dish gently. The whole experiment was done in the chamber to avoid contamination.

7. Incubation

The Petri plates were incubated at 25°C for 18-24 hours for formation of colony.

8. Isolation

When the growth was visible on the media, the distinctly separated conidial colonics were isolated. A part of the well separated colony was cut agar surface by isolating needle and was inoculated into culturing test tubes containing VM media.

9. Incubation

The isolates were incubated at a temperature of 25°C for 3-4 days for the proper growth of conidiation of the inoculates.

10. Classification

The cultures were observed after 5 days and classified by comparing their characters with wild type Em'a'

STUDIES ON MUTAGENIC EFFECT OF LEAF EXTRCT OF *Asparagus recemosus* Linn. ON THE GROWTH OF *Neurospora crassa*

Preparation of solution of concentration of *Asparagus recemosus* Linn.

At first 50 gm of fresh raw leaves of *Asparagus recemosus* Linn. were measured with the help of a balance . The leaves were washed with tap water 2-3 times and finally it was washed with distilled water and air dried. A paste was made with the help of a electric grinder. The paste was filtered 2-3 times with the help of sterilized thick cotton and finally it was filtered with the help of sterilized filter paper.The whole filtration was done in a inoculation chamber to prevent contamination. The concentration was divided as D_O, D_1, D_2, D_3 and D_4 with VM & 1ml distilled water,1,2,4 dorps and 1ml leaf extract respectively. Each concentration taken in duplicates and one set was sterilized in the autoclave at 121°C under 15 lb pressure for 20 minutes and other set was kept at normal temperature of the laboratory.

EFFECT OF NRORMAL(UNAUTOCLAVED) AND AUTOCLAVED LEAF EXTRACT OF *Asparagus recemosus* Willd. ON THE GROWTH OF *Neurospora crassa*:

At first the Petri dishes were marked as 0, 1, 2,4drops and 1ml with the help of glass marker. 1,2,4 drops and 1ml of normal and autoclaved D_1,D_2,D_3 and D_4 concentration were taken in separate sterilized Petridis with the help of sterilized pipette. 10 ml of molten VM plating medium was added in each Petridis and the Petri dishes were shaken gently for thorough mixing of the extracts. As for control VM plating medium was taken in each sterilized Petridis. When the Petridis was cooled 4 days old Em'a' was inoculated at the centre of the Petri dishes inside the inoculation chamber to prevent contamination. Test for both control and the leaf extract concentration was done in duplicate. The Petri dishes were kept in the incubator at 25°C and observed after17,23,39 and 46 hours.

TREATING OF Em 'a' WITH EXTRACT OF LEAF OF *Asparagus recemosus* Linn.

1.Obtaining of fresh culture Em'a'

For obtaining of fresh culture Em'a' was sub cultured 4 times after an interval of 4 days. In each case culture of 5 days old were used for treating coinidia.

2. Sterilization

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

3. Centrifugation

8 ml of *Daturine leaf extract* of the concentration D1, D3, D4 and D5 were taken into for centrifuge tubes. 1 loop of conidia of Em'a' was taken into each tube and was shaken for homogeneous solution. The solution was ce4ntrifuged with the help of a centrifuge machine for 15-20 minutes.

4. Filtration

The solution of the centrifuge tubes were poured out carefully. So the conidia could remain in the bottoms of the tubes. 8 ml of sterilized distilled water was taken into each tube and centrifuged for 3 minutes. The upper liquid was poured out. The same procedure of pouring out upper liquid, adding water and centrifugation was repeated 2 times.

5. Preparation of suspension with treated conidia

8 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 drop of the suspension was taken in each sterilized Petridis. The Petridis was marked asD_1, D_2, D_4 and D_5 previously and the suspension was taken accordingly. 10 ml of molten SM at 47°C was added to each Petridis. The temperature of the media did not exceed 47°C. The suspension and media were mixed well by shaking the Petridis gently.

The whole experiment was done in the chamber to avoid contamination.

7. Incubation

The Petridis were incubated in an incubator at 25°C.

8. Isolation

When the growth was visible on the media, the distinctly separated conidial colonics were isolated. A part of the well separated colony was cut 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°C.

10. Classification

The cultures were observed after 5 days and classified by comparing their characters with wild type Em'a'.

ARTIFICIAL INDUCTION OF MUTATION BY UV IRRADIATION.

1. Obtaining of fresh culture

Emerson'a' was used for treating conidia. To obtain fresh culture of Em'a' it was subcultured 4 times after 4 days intervals in every time.

2. Preparation of conidial suspension

At first 3 small size, sterilized Petri dishes were marked as1,2 and 3. 20 ml of sterilized distilled water was taken in a large sterilized test tube and added 3 loops of conidia from 5 days old Em'a'. The tube was shaken gently for homogenous conidial suspension.The conidial suspension was filtered with the help of sterilized funnel through absorbing cotton layers. 5 ml of conidial suspension were taken in each of the petridish no 1, 2 and 3.

3. Irradiation

The UV lamp was set up at a height of 15 cm. on a table in the dark room. Then height was made on. After 1 hour Petridishes no. 1, 2 and 3 with conidial suspension were respectively for 120 seconds, 150 seconds and 180 seconds respectively with UV light. Radiation was given at a distance of 15 cm. The plate with the suspension was continuously rotated under UV light with a wooden handle to ensure an equal dose of the suspension. After completion of the irradiation, the suspension was stored in the dark for 2 hours to prevent "photo-reactivation".

The most effective wavelength for radiation is 2540 A which is absorbed by DNA. In the Genetic Research Laboratory, the conidia were exposed to the wavelength of 254 nm (i.e. 2540 A).

50% of each irradiated conidial suspension was in a separate sterilized radiation tube (8"×1") fitted with pipette. The radiation tubes were marked previously. Then the irradiation conidia were plated in the following ways.

A. The UV irradiation conidial suspension was plated with molten SM containing leucine, lysine arginine,adenine,histidine and tryptophan.

B. Repeated filtation

5 ml sterilized VM liquid media was added in each radiation tube and fitted in the water bath at 25°C. The plastic tubing of air were fitted with pipettes of the radiation tube and the water pump was started. The continuous sterile ai9r was passed through the suspension for gentle stirring and repeated filtration was done after every 8 hours.

C.Plating

After 96 hours the irradiated suspension was plated with molten SM containing leucinine,lysine, adenine, histidine and tryptophan.

4. Incubation

The plates were incubated at 25°C for growth of conidial colony.

5. Isolation

When the growth was invisible on the media the separated conidial colonics were isolated. A part of the well separated colony was cut with agar with the help of isolating needle and was inoculated into VM tubes containing leucinine,lysine, adenine, histidine and tryptophan.

6. Incubation

The cultures were incubated at 25°C for growth of mycelia and conidia.

7. Observation and Classification

The cultures were observed after 5 days and classified by comparing there characters with wild type Em'a".

Biochemical test

All the isolates were tested on SM and. Those cultures which did not grow on SM, but grew on supplemented SM, they were the biochemical mutants.

SELECTION OF SOME MORPHOLOGICAL MUTANTS

Mutants from different groups were subcultured 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, mycelial groth,growth pattern, germination, crossing and linkage.

CROSSING

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

Westergaard's crossing medium was usually used for crossing. In cases wrere the crosses were strile .i.e. did not form perithecia then Suyama's crossing medium was used. Crossing was done in

large tube (6") containing a 'W' shaped filter paper and 10-12ml. of Westergaard's medium. One strain was put in one trough of the 'W' shaped filter paper plant and the opposite strain was put on another trough. The crosses were then incubated at 25°C. The fertile cultures from perithecia in about 6-10 days. The morphological mutants were crossed with wild types EM 'a' and EM 'A' for checking mating types and they were crossed with biochemical markers of seven linkage groups for the study of linkage.

It was observed that the more delay in formation of perithecia, the less in the number of perithesia fromed in a cross.

Further,the greater the concentration of amino acid supplement the earlier was the formation of perithecia and also the higher the number of perithecia. The perithecia generally liberated spores in the 2^{nd} week but occasionally in the 3^{rd} week.

If the crosses were sterile, the quantily of sugar in the medium reduced to 0.2% and the amino acid supplement was increased to 50mg per 100ml of the medium in accordance with the findings of Suyama et al.(1958).

Improvement of fertility of the mutants

1. The mutants were subcultured four times with 4 days interval to improve their fertility
2. Rapid subcultured mutants were crossed in Westergaard's medium containing 50 mg percent amino acid with the wild types or the marker.
3. The wild types or the markers as the case might be, were subcultured four times before crosses were made.
4. For good conidia formation, the concentration of tryptophan was used 40mg per 100 ml media.
5. The crosses were kept at 25°C bath.

STUDY OF SEGREGATION AND LINKAGE

When the crosses between mutant and wild had formed perithecia and the spores had shed 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 tube and ther 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 mutant isolates and wild type isolates was calculated. In most casea, it was found to be about 1:1.

Then a number of isolates from two groups (mutant and wild type isolates) were crossed with EM 'a' and EM 'A'. The isolates that formed perithecia with EM 'a' was the strain- 'A' and the strain which formed perithecia with EM 'a' and 'A' was about 1:1.

For the study of segregation the following procedure was followed:

1. Spreading of spores.
2. Isolation of single spores.
3. Incubation of the isolates.
4. Analysis of the isolates.
5. Determination of the mating types of the isolates.

1. Spreading of spores

When plenty of spores had shed the cross was ready for spreading. A spreading needle made of glass rod bend twice at right angles was boiled for 20 minutes. 2-3 drops of sterilizer distilled water was put on SM plates with sterile pipette. Some places on the crossing tube with sproes were marked with the help of glass marking pencile under microscope. A wire loop needle was burnt red heat and cooled in sterilizer 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 transferd to the distilled water droup on SM plate. The sterilized spreading needle was then cooled in sterilized distilled water plate and the water with spores was spreaded uniformaly 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°C for 50 minutes and then incubated at 25°C for 12-16 hours as necessary.

2. Isolation of single spores

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

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

3. Incubation

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

4. Analysis of the isolates

After 4to 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.

5. Determinatio of the mating type of the isolates

The selected isolates were chosen and their mating types were determined by the crossing them with the wild type of both Em'a' and Em'A'. If perithesia formed with Em'a' the mating type of the extract would be 'A' and if perithecia formed with Em'A' it would be 'a'.

COMPARATIVE STUDY OF MYCELIALGROWTH:

To study mycelial growth, mutants and wild types of *Neurospora crassa* werecultured in liquid Vogel's minimal 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 was taken first. When the culture was 72 hours old flasks with cultures were heated in water in2minutesand were filtered with filter papers of known weight. The filter papers with mycelia were dried for two day in the oven. Precaution was taken so that the cultures did not burn. Then the weight of filter papers with culture taken. The actual result was found by subtracting the weight of the empty filter paper from the weight of the filter paper containing dried mycelia.

STUDY OF LINEAR GROWTH

The cultures inoculated in race tubes. The race tubes were made of long Pyrex glass tubes. The horizontal portion was kept 40 cm with an internal diameter of about 14 mm. One side of the race tube was bent up at an angle of 45°C and the opposite side at 90° angle. The opening was fire polished and flared slightly.

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

CHAPTER - 5

EXPERIMENT AND RESULTS

TO FIND OUT IF THERE WAS ANY SPONTANEOUS MUTATION IN *Neurospora crassa*

Treating of Em'a'

Conidia from Em'a' of 4 days old were treated with sterilized distilled water for 10 minutes. SM was used as plating media. After 16 hours about 4,000 single conidial colonics were isolated.

Observation

The cultures incubated at 25°C for growth of mycelia and conidia. After 5 days the isolates were observed if there were any abnormalities in their characters by comparing with that of Em'a'.

Conclusion

Among those 4,000 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.

EFFECT OF NORMAL(UNAUTOCLAVED) AND AUTOCLAVED LEAF EXTRACT OF *Datura metel* Linn. ON THE GROWTH OF *Neurospora crassa*

The Petridis were marked as 0drop (control),1 drop,2 drops,4 drops and 1 ml of normal (unautoclaved) and autoclaved D_0,D_1,D_2,D_3 and D_4 concentration were taken in each sterilized Petri dish. 10 ml of molten VM plating media was added to each Petri dish. When the Petri dish was cooled, Em'a' was inoculated to the center of the Petridis. The Petri dish was kept in the incubator at 25°C and observed after 18, 24, 30, 36 and 42 hours and measured the radial growth of the colony was in centimeter. The results were given in Table-1, 2 and 3.

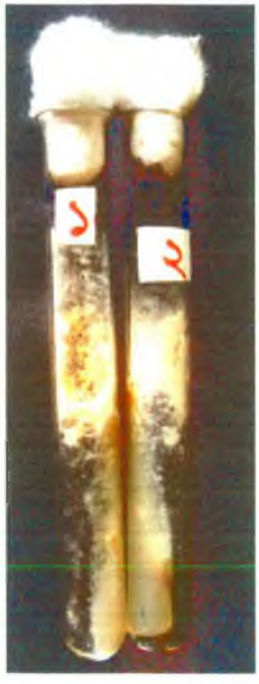


Fig.1 wild: type a) Em'a' , b) Em'A'

Table -1

Different doses of leaf extract of *Datura metel* Linn.

Serial number	Amount of leaf extract	Name of the concentration
1	8 ml	D_0
2	8 ml	D_1
3	8 ml	D_2
4	8 ml	D_3
5	8 ml	D_4

Table -2

Effect of non-sterilized normal concentration of leaf extract of *Datura metel* Linn. on the growth of *Neurospora crassa*:

Observation after the following hours	Different doses of non sterilized normal concentration									
	0 drop		1 drop		2 drops		4 drops		1 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
18	1.9 2.0	1.95	0.2 0.5	0.35	0.5 0.6	0.55	0.8 0.8	0.8	0.3 0.4	0.35
24	2.5 2.7	2.6	0.7 0.6	0.65	0.9 1.5	1.2	1.0 1.1	1.05	0.5 0.6	0.55
30	3.5 3.9	3.7	1.3 1.5	1.4	1.4 1.7	1.55	1.4 1.5	1.45	1.2 1.1	1.15
36	4.5 4.5	4.5	1.7 1.6	1.65	1.8 2.0	1.9	2.2 2.4	2.3	1.6 1.6	1.6
42	5.2 5.4	5.3	1.9 2.0	1.95	2.0 2.1	2.05	2.9 2.8	2.85	1.8 1.9	1.85

Table -3

Effect of sterilized concentration of leaf extract of *Daetura metel* Linn. on the growth of *Neurospora crassa*:

Observation after the following hours	Different doses of non sterilized normal concentration									
	0 drop		1 drop		2 drops		4 drops		1 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
18	1.9 2.0	1.95	1.8 1.9	1.85	1.6 1.5	1.55	1.7 1.6	1.65	1.1 1.0	1.05
24	2.5 2.7	2.6	2.4 2.3	2.35	1.9 2.0	1.95	2.2 2.1	2.15	1.6 1.5	1.55
30	3.5 3.9	3.7	3.4 3.3	3.35	2.9 3.0	2.95	3.7 3.5	3.6	2.6 2.4	2.5
36	4.5 4.5	4.5	4.9 4.0	4.45	3.4 3.2	3.3	4.0 3.9	3.95	3.0 2.8	2.9
42	5.2 5.4	5.3	5.1 4.8	4.85	3.6 3.4	3.5	4.2 4.1	4.15	3.2 2.9	3.05

Conclution

From table 2 and 3 it was found that, the concentration of leaf extract of *Datura metel* Linn. Control the radial growth of wild type *Neurospora crassa* . The result also showed that non sterilized concentration was more effective than sterilized one. Non sterilized D_4 concentration was the most effective and sterilized D3 concentration was the least effective.

So, non sterilized D_4 concentration of leaf extract of *Datura metel* Linn. were selected for treating wild type (Em'a') conidia.

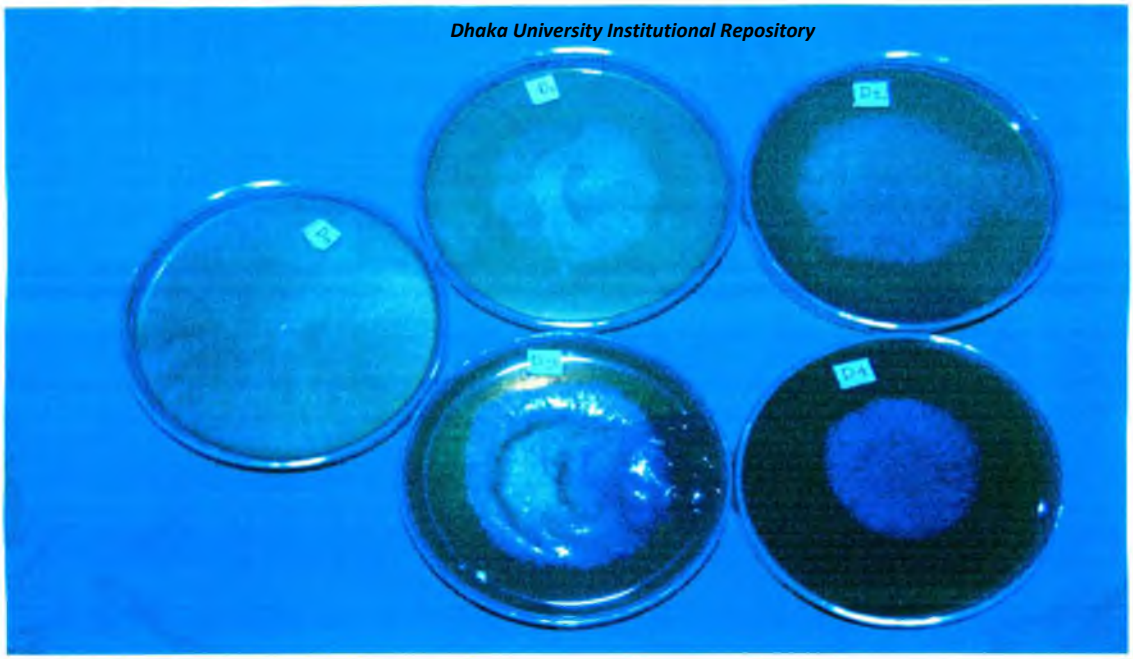


Fig-2 Effect of different doses of non sterilized normal concentration of *Detura metel* Linn. on the growth of N. crassa. D_0VM, D_1 1- drop, D_2 2- drops, D_3 4- drops, D_4 1- ml,

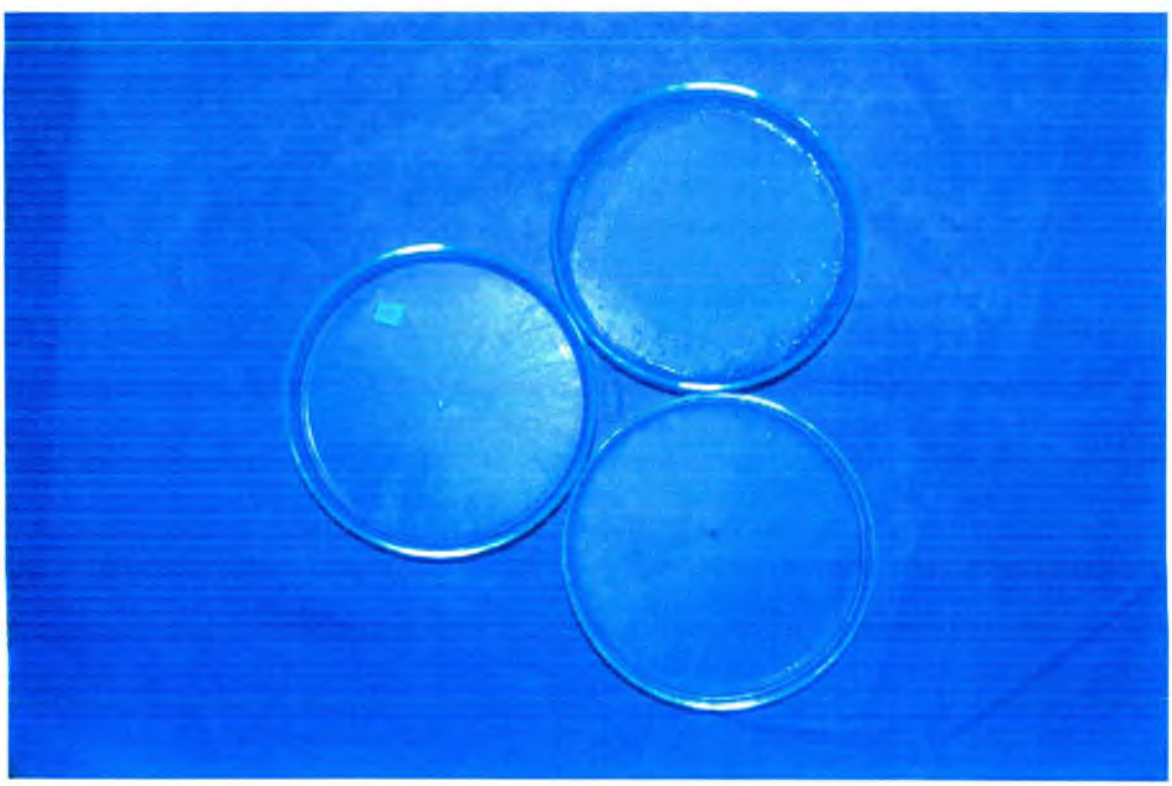


Fig-3 Effect of different doses of sterilized concentration of Leaf extract of *Detura metel* Linn. on the growth of N. crassa. D_0VM, D_1 1- drop,D_2 2- drops, D_3 4- drops, D_4 1- ml,

INDUCTION OF MUTATION WITH LEAF EXTRCT (*Datura metel* Linn.) SOLUTION OF THE CONCENTRATION D_4

Conidia of Em'a' of 5 days old culture was treated with leaf extract(*Datura metel* L.) concentration of D_4 for 10 minutes. SM was used plating and VM in small tubes was used as culturing media. Plates with treated conidia were incubated at 25C. Single conidial colonics were isolated after 48 hours of incubation. Then the culture was incubated at 25C and observed after 5 days. The culture that shows abnormality with Em'a' of same age was denoted as mutants. The mutants were different from Em'a' in colour of cinidia mode of conidia mycelial structure etc.

Classification and nomenclature of obtained mutants induced with extract of leaf of *Datura metel* Linn.

Group A: *plug*

The mycelial growth inside the tube, deep pink conidia formed outside the tube, conidia around plug. A single mutant fall under this group.

D_4 -900, 905

Group-B: *vigorous*

Pink coloured profuse conidial growth which reaches upto the plug. The following 15 mutants fall in this group.

D_4 -718,719, D_4- 1003, 1004, 1005, 1006, 1007, 1008, 1009

D_4- 401, 411, D_4-859, 860, 911.

Group C: *ropy*

Mycelia form a rope like structure,mycelia colourless,growth less than wild type condia pink.
The following 10 mutants fall into this group :
1210,1211,1212,1213,1214,1215,1216,1217,1218,1219.

Group D: *fluffy*

Mycelia are colourless conidia are absent or very few in number, mycelia are cottony and growth is vigorous. The following 13 mutants fall into this group.

D_4-771,772,773,774,775,953,954,955,956,957,958,1103,1104.
Group E: *albino*

Mycelia and conidia are colourless. The following 5 mutants fall into this group.

D_4-628,962,963,1001,1002.

Group F : *conidial lump*
. *conidial lump* is a pink coloured lumped structure. A densed pink conidial lumped structure.

D_4- 524,765

Table-4

Classification of obtained mutants induced with leaf extract of *Datura metel* Linn

Name of roup	Characteristics	Concentration of the leaf extract	No. of mutant	Name of the mutant
A	Conidia formed outside the plug	D_4	2	*plug*
B	Huge conidial growth that touches the plug	D_4	15	*vigorous*
C	Mycelia rope like	D_4	15	*ropy*
D	Profuse mycelial growth	D_4	13	*fluffy*
E	Colourless mycelia and cinidia	D_4	5	*albino*

EFFECT OF NORMAL(UNAUTOCLAVED) AND AUTOCLAVED LEAF EXTRACT OF *Eclipta prostrata* Linn. ON THE GROWTH OF *Neurospora crassa*:

The petridish were marked as 0drop(control),1 drop,2 drops,4 drops and 1 ml of normal (unautoclaved) and autoclaved Eo,E_1,E_2,E_3 and E_4 concentration were taken in each sterilized petridish. 10 ml of molten VM plating media was added to each petridish. When the petridish was cooled, Em'a' was inoculatedto the center of the petridish. The Petridish was kept in the incubator at 25°C and observed after 18,24,30, 36 and 42 hours and measured the radial growth of the colonywas in centimeter. The results were given in Table-5, 6 and 7.

Table -5

Different doses of leaf extract of ***Eclipta prostrata*** Linn.

Serial number	Amount of leaf extract	Name of the concentration
1	8 ml	E_0
2	8 ml	E_1
3	8 ml	E_2
4	8 ml	E_3
5	8 ml	E_4

Table-6

Effect of non-sterilized normal concentration of leaf extract of *Eclipta prostrata* Linn.on the growth of *Neurospora crassa*:

Observation after the following hours	Different doses of non sterilized normal concentration									
	0 drop		1 drop		2 drops		4 drops		1 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
18	1.9 2.0	1.95	0.2 0.3	0.25	0.4 0.6	0.5	0.3 0.4	0.35	Θ	Θ
24	2.5 2.7	2.6	1.2 1.5	1.35	1.1 1.2	1.15	1.0 1.1	1.05	Θ	Θ
30	3.5 3.9	3.7	1.4 1.6	1.5	1.3 1.4	1.35	1.2 1.4	1.3	0.9 0.8	0.85
36	4.5 4.5	4.5	1.7 1.8	1.75	1.4 1.5	1.45	1.5 1.8	1.65	1.0 1.0	1.0
42	5.2 5.4	5.3	2.8 2.9	2.85	1.6 1.7	1.65	1.7 2.2	1.95	1.1 1.2	1.15

Note: O indicates no growth
Θ indicates some growth

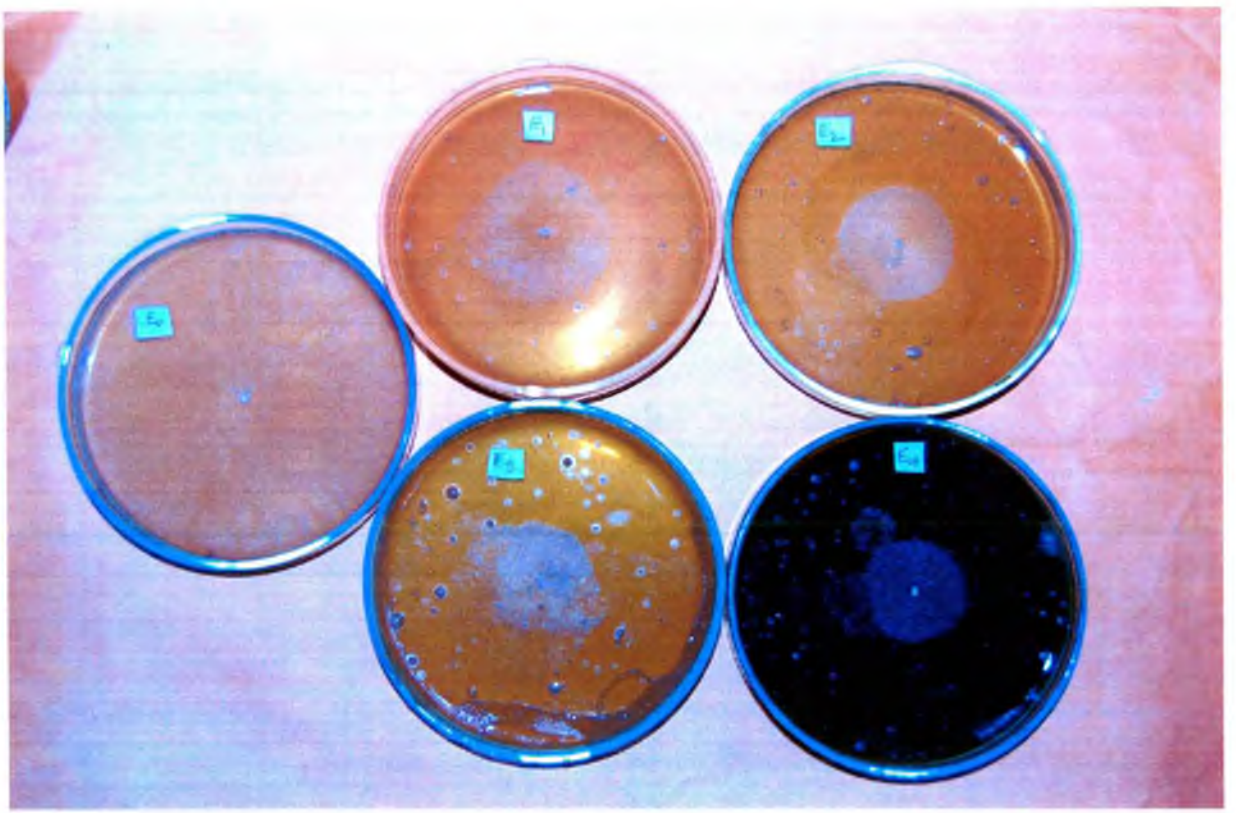


Fig-4 Effect of different doses of non-sterilized normal concentration of Leaf extract of *Eclipta prostrata* Linn. on the growth of N. crassa. D_0 VM, D_1 1- drop, D_2 2- drops, D_3 4- drops, D_4 1- ml,

Table -7

Effect of sterilized concentration of leaf extract of *Eclipta prostrata* Linn.. on the growth of *Neurospora crassa*:

Observation after the following hours	Different doses of non sterilized normal concentration									
	0 drop		1 drop		2 drops		4 drops		1 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
18	1.9 2.0	1.95	1.8 1.9	1.85	1.6 1.5	1.55	1.7 1.6	1.65	1.1 1.0	1.05
24	2.5 2.7	2.6	2.4 2.3	2.35	1.9 2.0	1.95	2.2 2.1	2.15	1.6 1.5	1.55
30	3.5 3.9	3.7	3.4 3.3	3.35	2.9 3.0	2.95	3.7 3.5	3.6	2.6 2.4	2.5
36	4.5 4.5	4.5	4.9 4.0	4.45	3.4 3.2	3.3	4.0 3.9	3.95	3.0 2.8	2.9
42	5.2 5.4	5.3	5.1 4.8	4.85	3.6 3.4	3.5	4.2 4.1	4.15	3.2 2.9	3.05

Conclution

From table 2 and 3 it was found that, the concentration of leaf extract of *Datura metel* Linn.
Control the radial growth of wild type *Neurospora crassa* . The result also showed that non sterilized concentration was more effective than sterilized one. Non sterilized D_4 concentration was the most effective and sterilized D3 concentration was the least effective.

So, non sterilized D_4 concentration of leaf extract of *Datura metel* Linn. were selected for treating wild type (Em'a') conidia.

INDUCTION OF MUTATION WITH LEAF EXTRCT (*Datura metel* Linn.) SOLUTION OF THE CONCENTRATION D4

Conidia of Em'a' of 5 days old culture was treated with leaf extract(*Datura metel* L.) concentration of D_4 for 10 minutes. SM was used plating and VM in small tubes was used as culturing media. Plates with treated conidia were incubated at 25C. Single conidial colonics were isolated after 48 hours of incubation. Then the culture was incubated at 25C and observed after 5 days. The culture that shows abnormality with Em'a' of same age was denoted as mutants. The mutants were different from Em'a' in colour of cinidia mode of conidia mycelial structure etc.

Classification and nomenclature of obtained mutants induced with extract of leaf of *Datura metel* Linn.

Group A: plug

The mycelial growth inside the tube, deep pink conidia formed outside the tube, conidia around plug. A single mutant fall under this group.

D_4 -900, 905

Group-B: vigorous

Pink coloured profuse conidial growth which reaches upto the plug. The following 15 mutants fall in this group.

D_4 -718,719, D_4- 1003, 1004, 1005, 1006, 1007, 1008, 1009

D_4- 401,411, D4-859, 860, 911.

Group C: ropy

Growth is checked. The hyphae are colourless and look like ropes, conidia orange and less in number 15 mutants of this group are as follows:
D_{4-} 483,487,488,522,523,524,525,658,659,660,661,762,763,764,765.

Group D: fluffy

Mycelia are colourless conidia are absent or very few in number, mycelia are cottony and growth is vigorous. The following 13 mutants fall into this group.
D_4-771,772,773,774,775,953,954,955,956,957,958,1103,1104.

Group E: albino

Mycelia and cinidia are colourless. The following 5 mutants fall into this group.

D_4-628, 962, 963, 1001, 1002.

Table-8

Classification of obtained mutants induced with leaf extract of *Eclipta prostrata* Linn.

Name of the group	Characteristics	Concentration of the leaf extract	No. of mutant	Name of the mutant
A	Conidia formed outside the plug	E_0	2	plug
B	Huge conidial growth that touches the plug	E_1	15	vigorous
C	Mycelia rope like	E_2	15	ropy
D	Profuse mycelial growth	E_3	13	fluffy
E	Colourless mycelia and cinidia	E_4	5	albino

EFFECT OF NORMAL(UNAUTOCLAVED) AND AUTOCLAVED Rhizome extract OF of *Asparagus racemosus* Linn. ON THE GROWTH OF *Neurospora crassa*:

The petridish were marked as 0drop (control),1 drop,2 drops,4 drops and 1 ml of normal (unautoclaved) and autoclaved A_0,A_1,A_2,A_3 and A_4 concentration were taken in each sterilized petridish. 10 ml of molten VM plating media was added to each petridish. When the petridish was cooled, Em'a' was inoculatedto the center of the petridish. The Petridish was kept in the incubator at 25°C and observed after 18, 24, 30, 36 and 42 hours and measured the radial growth of the colonywas in centimeter. The results were given in Table-1, 2 and 3.

Table -9

Different doses of leaf extract of *Asparagus racemosus* Linn.

Serial number	Amount of leaf extract	Name of the concentration
1	8 ml	A_0
2	8 ml	A_1
3	8 ml	A_2
4	8 ml	A_3
5	8 ml	A_4

Table -10

Effect of non-sterilized normal concentration of Rhizome extract of *Asparagus racemosus* Linn. on the growth of *Neurospora crassa*:

Observation after the following hours	Different doses of non sterilized normal concentration									
	0 drop		1 drop		2 drops		4 drops		1 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
18	1.9 2.0	1.95	1.2 0.9	1.05	0.3 0.3	0.3	0.2 0.3	0.25	0.2 0.3	0.25
24	2.5 2.7	2.6	2.2 2.7	2.45	0.5 0.6	0.55	0.8 0.9	0.85	0.4 0.6	0.5
30	3.5 3.9	3.7	2.6 2.8	2.7	0.9 1.0	0.95	1.1 1.3	1.2	0.8 0.9	0.85
36	4.5 4.5	4.5	2.9 2.8	2.85	1.9 2.0	1.94	1.6 1.7	1.65	1.1 1.2	1.15
42	5.2 5.4	5.3	3.2 3.3	3.25	1.9 2.0	1.95	2.1 2.2	2.15	1.6 1.8	1.7

Table -11

Effect of sterilized normal concentration of Rhizome extract of *Asparagus racemosus* Linn. on the growth of *Neurospora crassa*

Observation after the following hours	Different doses of non sterilized normal concentration									
	0 drop		1 drop		2 drops		4 drops		1 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
18	1.9 2.0	1.95	1.8 1.9	1.85	1.6 1.5	1.55	1.7 1.6	1.65	1.1 1.0	1.05
24	2.5 2.7	2.6	2.4 2.3	2.35	1.9 2.0	1.95	2.2 2.1	2.15	1.6 1.5	1.55
30	3.5 3.9	3.7	3.4 3.3	3.35	2.9 3.0	2.95	3.7 3.5	3.6	2.6 2.4	2.5
36	4.5 4.5	4.5	4.9 4.0	4.45	3.4 3.2	3.3	4.0 3.9	3.95	3.0 2.8	2.9
42	5.2 5.4	5.3	5.1 4.8	4.85	3.6 3.4	3.5	4.2 4.1	4.15	3.2 2.9	3.05

Conclusion

From table 2 and 3 it was found that, the concentration of leaf extract of *Asparagus racemosus* Linn . Control the radial growth of wild type *Nurospora crassa* . The result also showed that non sterilized concentration was more effective than sterilized one. Non sterilized D4 concentration was the most effective and sterilized D3 concentration was the least effective.

So, none sterilized A_4 concentration of leaf extract of *Asparagus racemosus* Willd. were selected for treating wild type (Em'a') conidia.

INDUCTION OF MUTATION WITH LEAF EXTRCT(*Asparagus racemosus* Linn) SOLUTION OF THE CONCENTRATION A_4

Conidia of Em'a' of 5 days old culture was treated with leaf extract (*Asparagus racemosus* Linn . concentration of A4 for 10 minutes. SM was used plating and VM in small tubes was used as culturing media. Plates with treated conidia were incubated at 25C. Single conidial colonics were isolated after 48 hours of incubation. Then the culture was incubated at 25C and observed after 5 days. The culture that shows abnormality with Em'a' of same age was denoted as mutants. The mutants were different from Em'a' in colour of cinidia mode of conidia mycelial structure etc.

Classification and nomenclature of obtained mutants induced with extract of leaf of *Asparagus racemosus* Linn.

Group A: *plug*

The mycelial growth inside the tube, deep pink conidia formed outside the tube, conidia around plug. A single mutant fall under this group.

A_4 -900, 905

Group-B: *vigorous*

Pink coloured profuse conidial growth which reaches upto the plug. The following 15 mutants fall in this group.

A_4 -718,719, A_4- 1003, 1004, 1005, 1006, 1007, 1008, 1009

A_4-401,411, A_4-859, 860, 911.

Group C: *ropy*

Growth is checked. The hyphae are colourless and look like ropes, conidia orange and less in number 15 mutants of this group are as follows:
A_4-483,487,488,522,523,524,525,658,659,660,661,762,763,764,765.

Group D: *fluffy*

Mycelia are colourless conidia are absent or very few in number, mycelia are cottony and growth is vigorous. The following 13 mutants fall into this group.
A_4-771,772,773,774,775,953,954,955,956,957,958,1103,1104.

Group E: *albino*

Mycelia and cinidia are colourless. The following 5 mutants fall into this group.

A_4-628,962,963,1001,1

Table-12

Classification of obtained mutants induced with leaf extract of *Asparagus racemosus* Linn.

Name of the group	Characteristics	Concentration of the leaf extract	No. of mutant	Name of the mutant
A	Conidia formed outside the plug	A_0	2	*plug*
B	Huge conidial growth that touches the plug	A_1	15	*vigorous*
C	Mycelia rope like	A_2	15	*ropy*
D	Profuse mycelial growth	A_3	13	*fluffy*
E	Colourless mycelia and cinidia	A_4	5	*albino*

Classification and nomenclature of obtained mutants induced with UV irradiation.

Group A: *plug*

The mycelial growth inside the tube, deep pink conidia formed outside the tube, conidia around plug. A single mutant fall under this group.

A_4 -900, 905

Group-B: *vigorous*

Pink coloured profuse conidial growth which reaches upto the plug. The following 15 mutants fall in this group.

A_4 -718,719, A_4- 1003, 1004, 1005, 1006, 1007, 1008, 1009

A_4-401,411, A_4-859, 860, 9

Group C: *ropy*

Growth is checked. The hyphae are colourless and look like ropes, conidia orange and less in number 15 mutants of this group are as follows:
A4-483,487,488,522,523,524,525,658,659,660,661,762,763,764,765.

Group D: *fluffy*

Mycelia are colourless conidia are absent or very few in number, mycelia are cottony and growth is vigorous. The following 13 mutants fall into this group.

Group E: *albino*

Mycelia and cinidia are colourless. The following 5 mutants fall into this group.

D_4-628,962,963,1001,1002.

A-771,772,773,774,775,953,954,955,956,957,958,1103,1104.

The following 5 mutants fall into this group.

Table-13

Classification of obtained mutants induced with UV irradiaton.

Name of the group	Characteristics of the mutants	Time of treatment	No. of mutants	Name of the mutant
A	Mycelia rope like	90 45	11 8	ropy
B	Both mycelia and conidia are colourless	90 45	5	albino
C	Growth less than wild	90 45	10 15	check
D	Colourless mycelia	45	8	fluffy
E	Profuse conidial growth touches the plug	90 45	3 6	vigorous

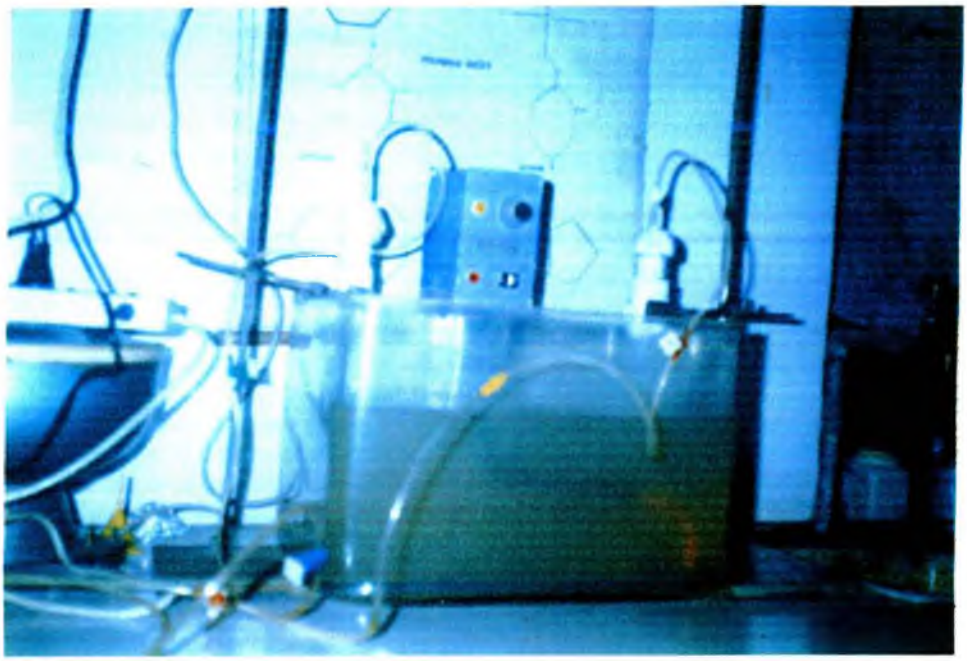


Fig. 7 filtration

Fig. 8 UV irradiation with morphological features of a) vigorous.b) ropy,c) albino,d) fluffy,

DETAILED STUDY OF SOME LEAF EXTRACT OF *Datura metel* Linn.,*Eclipta prostrata* Linn., RHIZOME EXTRACT OF *Asparagus racemosus* Linn. AND UV IRRADIATION INDUCED MUTANTS

Detailed study of some selected mutants induced with rhizome extract of *Asparagus recemosus* Linn.,leaf extract of *Datura metel* Linn. and UV irradiation.

Table-14

The following 9 mutants obtained from rhizome extract of *Asparagus recemosus* Wild.,leaf extract of *Detura metel* L. and UV irradiation were selected for further analysis.

No. of mutants	Name of the mutant
D_4-502	*check*
D_4-517	*ropy*
E_1-568	*Conidial band*
E_1-576	*vigorous*
E_1-30	*buff*
A_1-55	*fluffy*
A_1-100	*plug*
1223	*albino*
1502	*conidial band*

Fig : 6. Comparative morphological features of 1 Ema (wild type),
1. Ema (wild type) 3, *vigorous.4 ropy 5.buff 6.albino*
7.fluffy 8. Con.band 9. Con lump 10. Plug

Table-15

The time required for germination of Em'a' and some selected mutants induced with leaf extract of *Datura metel* Linn., *Eclipta prostrata* Linn., *Aspara gus recemosus* Linn. and UV Irradiation.

Name of the culture	Observation after the following hours								Inference on germination
	1	2	3	4	5	6	7	8	
Em'a'	Germination not started	Germination not started	Germination not started	Germination just started	Clear germination observed	Germination completed	Excellent germination observed		Em'a' germinates within 6 hours
fluffy	Germination not started	Germination not started	Germination not started	Germination not started	Conidia became swollen	Germination just started			Fluffy germinates within 8 hours
buff	Germination not started	Germination not started	Conidia became swollen	Clear germination observed	Germination completed				Buff germinates within 5 hours
plug	Germination not started	Germination not started	Germination not started	Germination just started	Exellent germination observed	Germination completed			Plug germinates within6 hours
albino	Germination not started	Germination not started	Germination not started	Germination not started	Conidia became swollen	Germination just started	Excellent germination observed	Germination completed	Albino germinates within 8 hours

ropy	Germination not started	Germination not started	Germination just started	Clear germination observed	Germination completed				Ropy germinates within 5 hours
conidial band	Germination not started	Germination not started	Conidia became rounded	Germination just started	Exellent germination observed	Exellent germination observed			Conidial band germinates within 6 hours
conidial lump	Germination not started	Germination not started	Germination not started	Germination not started	Germination just started	Clear germination observed	Germination completed		Conidial lump germinates within 7 hours
vigorous	Germination not started	Germination not started	Conidia became swollen	Clear germination observed	Germination completed				Vigorous germinates within 5 hours

Conclusion: The time required for germination of the mutants were deferent from that of wild type Em`a`

Table-16

Radial growth of Em'a' and some selected mutants induced with leaf extract of *Datura metel* Linn., *Eclipta prostrata* Linn., *Asparagus recemosus* Linn. and UV Irradiation.

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
Em'a'	1.5 1.8	1.65	2.6 3.0	2.8	3.3 3.6	3.45	4.0 4.1	4.05	5.0 5.0	5.0	over	over
vigorous	1.9 1.8	1.85	2.6 2.3	2.45	3.2 3.1	3.15	4.1 3.9	4.0	4.9 4.7	4.8	over	over
ropy	0.5 0.4	0.45	0.9 0.8	0.85	1.3 1.2	1.25	1.6 1.4	1.5	2.0 1.8	1.9	3.0 2.8	2.9
buff	0.6 0.5	0.55	1.0 1.0	1.0	1.7 1.6	1.65	1.9 1.8	1.85	2.6 2.5	2.55	3.2 3.1	3.15
albino	0.2 0.3	0.25	0.5 0.6	0.55	0.9 0.8	0.85	1.2 1.1	1.15	1.4 1.2	1.3	1.6 1.4	1.5
fluffy	0.3 0.6	0.45	0.5 0.9	0.7	0.9 1.4	1.15	1.3 1.6	1.45	1.7 1.9	1.8	2.0 2.1	2.05
conidial band	1.1 1.2	1.15	2.2 2.4	2.3	3.5 3.7	3.6	4.0 4.2	4.1	4.5 4.6	4.55	over	over
conidial lump	1.3 2.0	1.65	2.6 2.8	2.7	3.9 3.7	3.8	4.3 4.2	4.25	over	over	over	over
plug	1.5 2.4	1.45	2.5 2.3	2.4	3.8 3.6	3.7	5.0 5.0	5.0	over	over	over	over

Conclusion: The mutants showed variation in their radial growth with that of wild type Em'a'

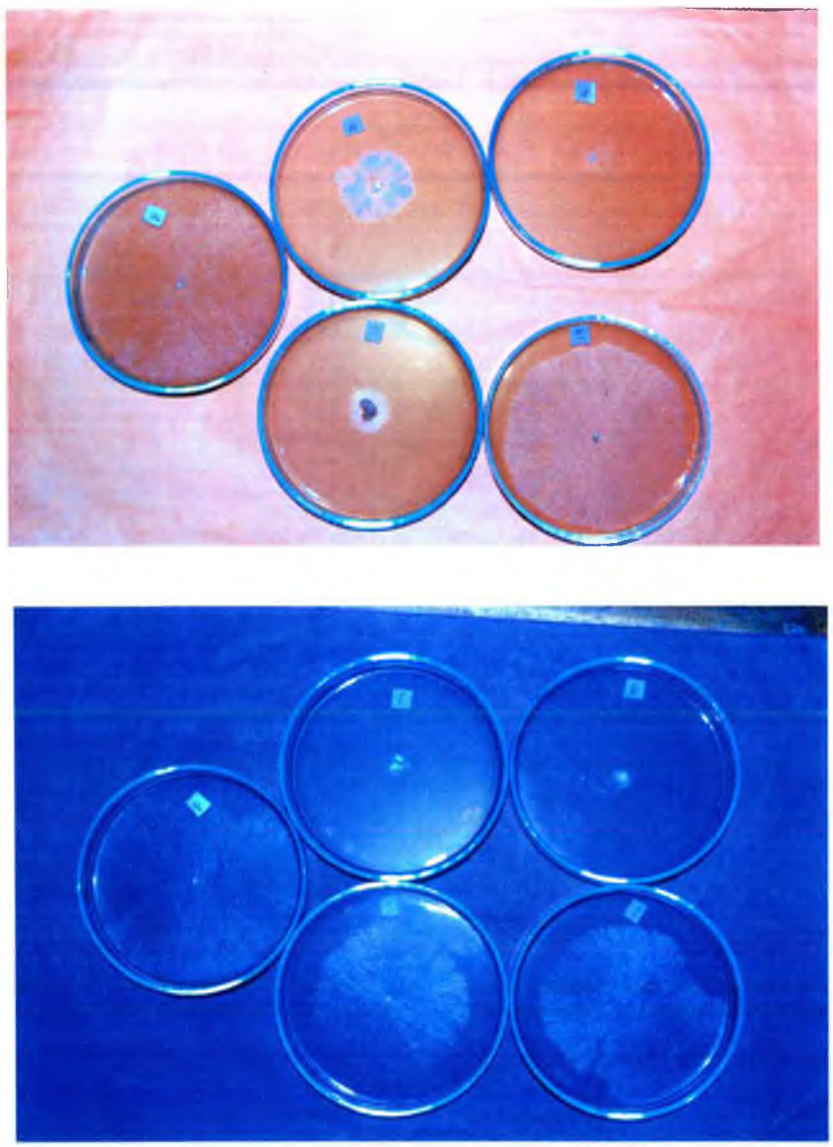


Fig:9. Radial growth of Em'a' and some selected mutants induced with leaf extract of Datura metel Linn., Eclipta prostrate Linn, Asparagus recemosus Linn. And UV Irradiation.

Table-17

Linear growth of Em'a' and some selected mutants induced with leaf extract of *Datura metel* Linn. Linn., *Eclipta prostrata* Linn., *Asparagus recemosus* Linn. and UV Irradiation.

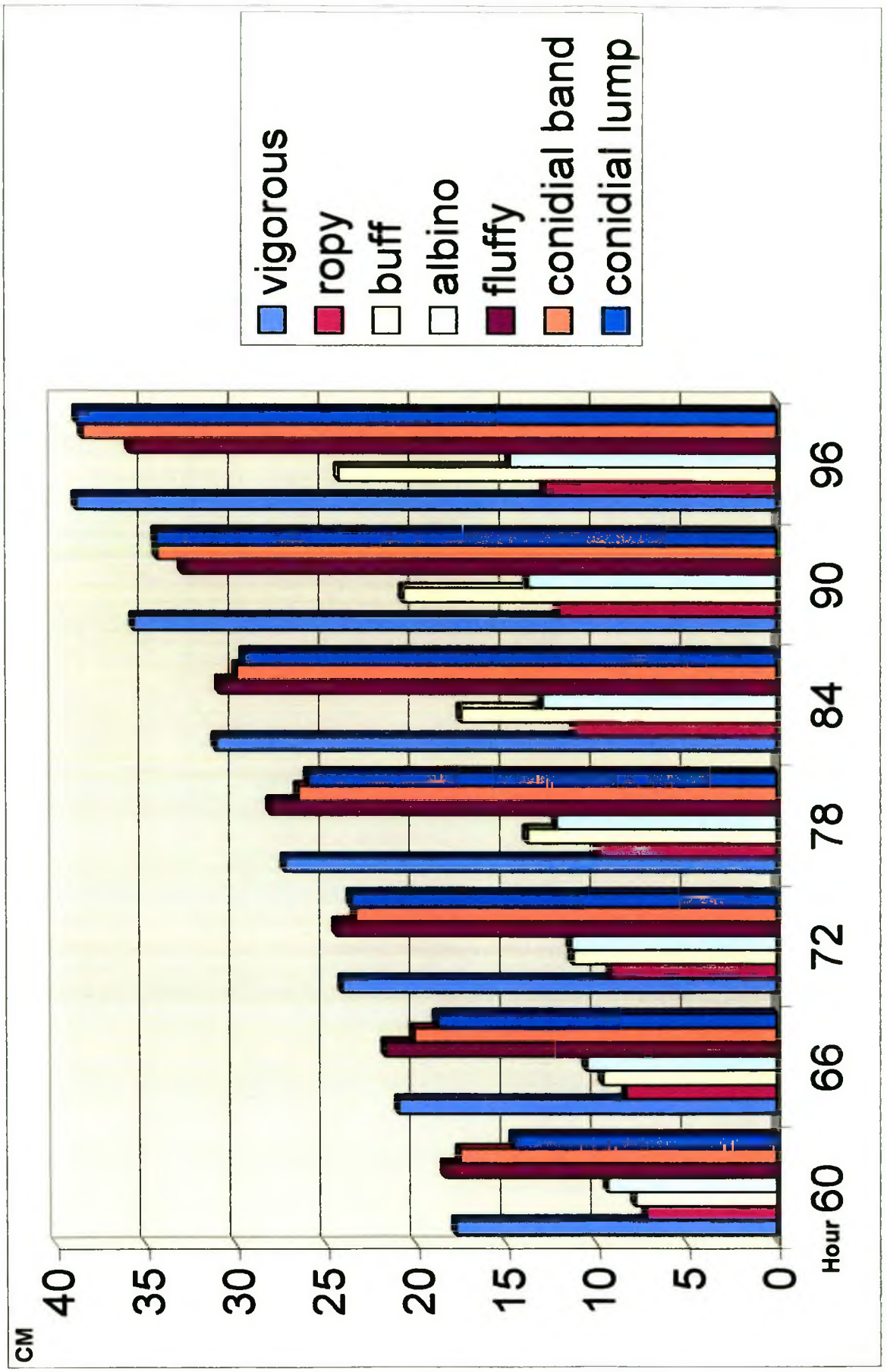
Name of the culture	Observation after the following hours													
	18		24		30		36		42		48		54	
	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm	Growth in cm	Mean in cm
Em'a'	1.9 2.0	1.95	2.8 3.0	2.9	4.9 5.2	5.05	6.7 6.9	6.8	8.5 8.8	8.65	10.3 10.9	10.6	11.4 12.0	11.7
vigorous	2.9 2.7	2.8	4.9 4.5	4.7	6.1 5.7	5.9	7.9 7.7	7.8	10.1 9.6	9.85	12.4 11.8	12.1	14.3 13.7	13.75
ropy	0.9 1.2	1.05	1.4 1.7	1.55	2.2 2.4	2.3	3.3 3.6	3.45	4.2 4.6	4.4	5.3 5.7	5.5	6.1 6.6	6.35
buff	1.0 1.2	1.1	1.9 2.1	2.0	2.7 2.9	2.8	3.6 4.0	3.8	4.9 5.3	5.1	5.7 6.1	5.8	6.6 6.7	6.65
albino	1.2 1.2	1.2	1.5 1.5	1.5	1.7 1.8	1.75	1.9 2.0	1.95	2.1 2.2	2.15	2.4 2.2	2.3	3.1 2.7	2.9
fluffy	2.1 2.3	2.2	3.4 3.7	3.55	5.0 5.4	5.2	7.1 7.6	7.35	9.4 9.9	9.65	12.0 12.6	12.3	15.7 16.1	15.8
conidial band	2.1 2.0	2.05	3.2 3.4	3.3	5.3 5.4	5.35	7.6 7.4	7.5	9.2 8.9	9.05	11.7 11.2	11.45	13.6 13.2	13.4
conidial lump	1.6 1.8	1.7	2.3 2.5	2.4	3.5 3.8	3.65	5.8 5.4	5.6	7.9 7.7	7.8	9.8 9.6	9.7	12.1 12.8	12.45
plug	3.5 3.2	3.35	6.2 6.0	6.1	9.1 94	9.25	12.5 12.9	12.7	15.1 15.7	15.8	18.7 19.2	18.95	22.2 22.8	22.5

Table-18

Linear growth of Em'a' and some selected mutants induced with leaf extract of *Datura metel* Linn., *Eclipta prostrata* Linn, *Asparagus recemosus* Linn. And UV Irradiation.

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
Em'a'	13.6 14.0	13.8	17.0 17.7	17.35	20.3 21.1	20.7	24.1 24.7	24.4	28.4 28.9	28.65	32.5 33.0	32.75	36.6 37.0	36.8
vigorous	18.0 17.6	17.8	21.2 20.7	20.95	24.8 23.4	24.1	27.5 27.0	27.25	31.3 30.8	31.05	35.8 35.4	35.6	38.9 38.6	38.75
ropy	7.2 7.3	7.25	8.4 8.7	8.35	9.0 9.4	9.2	9.8 10.0	9.9	11.1 11.3	11.2	12.0 12.1	12.05	12.7 12.9	12.8
buff	7.6 8.1	7.85	9.2 10.1	9.65	11.0 11.6	11.3	13.5 14.1	13.8	17.1 17.8	17.45	20.4 20.9	20.65	23.9 24.6	24.25
albino	9.7 9.1	9.4	10.8 10.3	10.55	11.7 11.1	11.4	12.5 11.9	12.2	13.2 12.7	12.95	14.1 13.9	13.75	15.3 14.7	14.75
fluffy	18.2 18.6	18.4	21.5 21.9	21.7	24.1 24.5	24.4	27.9 28.2	28.05	30.6 31.1	30.85	32.6 33.2	32.9	25.2 36.4	35.8
conidial band	17.7 17.4	17.55	20.3 19.9	20.1	23.6 23.1	23.35	26.7 26.3	26.5	30.1 29.7	29.9	34.5 34.1	34.3	38.8 38.0	38.4
conidial lump	14.8 14.4	14.6	19.0 18.6	18.8	23.9 23.3	23.6	26.2 25.7	25.95	29.8 29.2	29.5	34.7 34.0	34.35	38.9 38.5	38.7
plug	24.3 24.9	24.6	27.0 27.5	27.25	30.1 30.7	30.4	32.3 32.9	32.6	35.2 35.8	35.5	38.2 38.6	38.4	40.0 40.0	40.0

Conclusion: The linear growth of the mutants were different from that of wild type Em'a'.



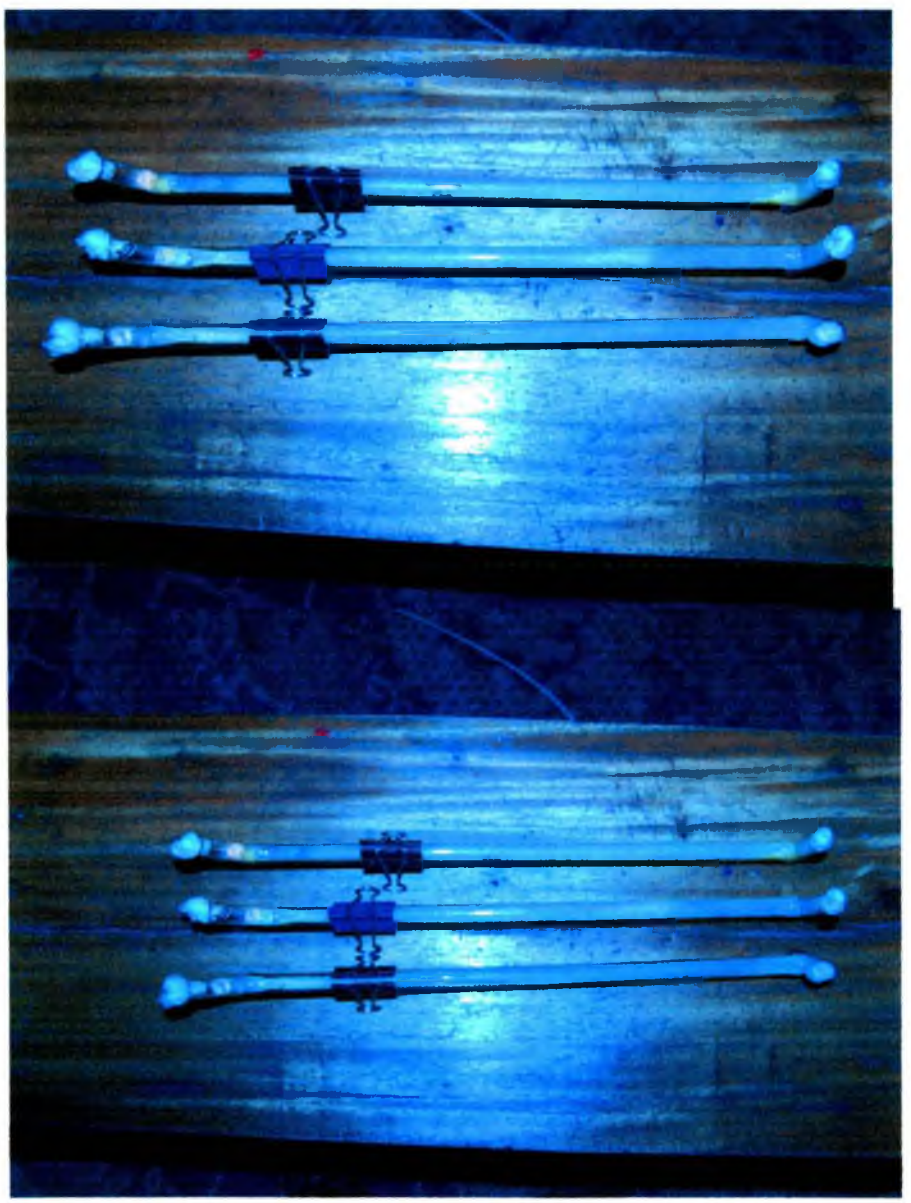


Fig : 10.Linear growth of Em' a' and some selected mutants 1. *vigorous 2. ropy 3. buff 4. albino 5.fluffy 6. plug.*

Table-19

Mycelia weight of Em'a' and some selected mutants induced with leaf extract of *Detura metel* Linn., *Eclipta prostrata* Linn., *Aspara gus recemosus* Linn. And UV Irradiation after 72 hours.

Number and name of the culture	Weight of the empty filter paper(g)	Weight of the filter paper containing dried mycelia(g)	Dry weight of the mycelia(g)	Mean(g)	Inference on mycelial growth
Em'a'	1.002 1.002	1.293 1.279	0.291 0.277	0.284	Normal
vigorous	1.002 1.002	1.325 1.323	0.323 0.321	0.322	Growth more than normal
ropy	1.002 1.002	1.172 1.149	0.170 0.147	0.159	Less than normal
buff	1.003 1.003	1.192 1.165	0.189 0.162	0.156	Growth less than normal
albino	1.002 1.002	1.124 1.135	0.122 0.133	0.128	Growth less than normal
fluffy	1.002 1.003	1.254 1.241	0.252 0.238	0.245	Growth less than normal
conidial band	1.002 1.002	1.287 1.345	0.285 0.343	0.314	Growth more than normal
conidial lump	1.003 1.003	1.261 1.242	0.258 0.239	0.249	Growth less than normal
Plug	1.002 1.002	1.384 1.357	0.382 0.355	0.369	Growth more than normal

Conclusion: It was observed in the above table that there was variation in the dry weight of selected mutants with Em'a

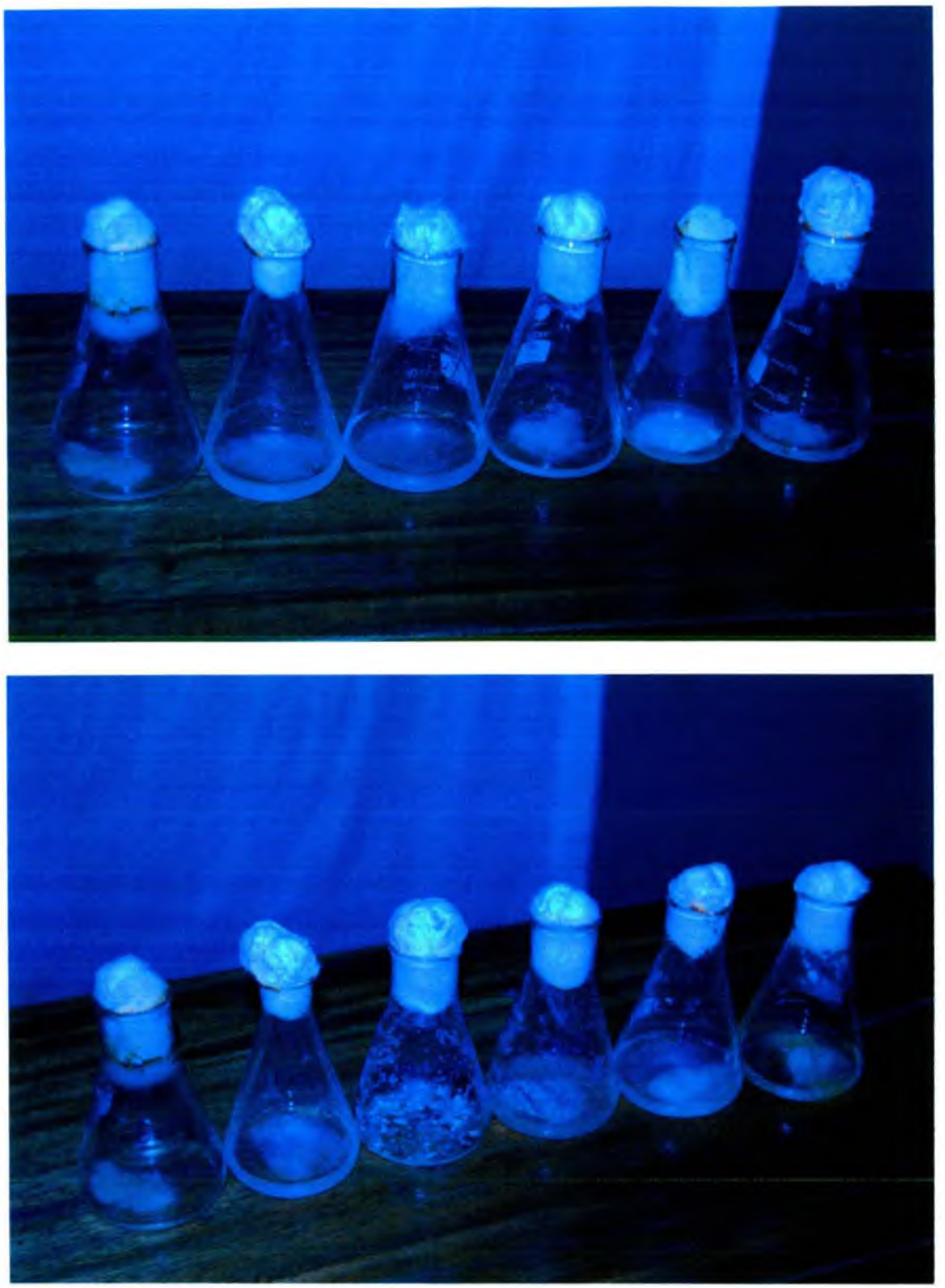


Fig: 11.Mycelia weight of Em' a'and Em'A' and some selected mutants induced with 1 vigorous 2. ropy 3. buff 4. albino 5.fluffy 6. Conidial band 7.conidial lump 8.plug

Table-20

Determination of mating type and fertility of the selected mutants induced with leaf extract of *Datura metel* L., *Eclipta prostrata* Linn., *Asparagus recemosus* Linn. And UV Irradiation.

All selected mutants were crossed with EM'a'and Em'A' separately observation was taken for 7-35 days.

Name of the culture	Designation of the cross	Whether perithecia formed yes/no	Perithecia formed in days	Size and number of perithecia	Spore shedding in days	Fertility	Mating type
vigorous	A1 576×Em'a'	No					
	A1×Em'A'	Yes	7	Large	28	Fertile	'a'
	D4 583× Em'a'	No					
	D4 583× Em'A'	yes	7	And many	27	Fertile	'a'
ropy	A1 515 × Em'a'	No					
	A1 515 × Em'A'	Yes	9	Medium	27	Fertile	'a'
	D4 770×Em'a'	No					
	D4 770×Em'A'	yes	10	And many	27	Fertile	'a'
buff	1030×Em'a'	No					
	1030×Em'A'	Yes	10	Medium	23	Fertile	'a'
	1051×Em'a'	No					
	1051×Em'A'	yes	12	And some	24	Fertile	'a'

albino	1222×Em'a'	No					
	1222×Em'A'	Yes	7	Large	23	Fertile	'a'
	1223×Em'a'	No					
	1223×Em'A'	yes	8	And some	24	Fertile	'a'
fluffy	E1 1155×Em'a'	No					
	E1 1155×Em'A'	Yes	15	Small	34	Fertile	'a'
	D4 1158×Em'a'	No					
	D4 1158×Em'A'	yes	15	And few	35	Fertile	'a'
conidial band	A1 568×Em'a'	No					
	A1 568×Em'A'	Yes	8	Large	31	Fertile	'a'
	D4 793×Em'a'	No					
	D4 793×Em'A'	yes	7	And many	33	Fertile	'a'
conidial lump	E1 540 Em'a'	No					
	E1 540×Em'A'	Yes	9	Large	24	Fertile	'a'
	D4 541 Em'a'	No					
	D4 541×Em'A'	yes	9	And many	23	Fertile	'a'
plug	D4 100 Em'a'	No		Large	20		
	D4 100 Em'A'	yes	7	And many	21	Fertile	'a'

Fig:12. Determination of mating type and fertility of All seleceted mutants were crossed with Em' a' and Em'A' s

Table-21

Segregating ratio of some selected mutants induced with leaf extract of *Datura metel* Linn., *Eclipta prostrata* Linn., *Asparagus recemosus* Linn. and UV Irradiation.

Name of the cross	No. of spores isolated	No. of wild	No. of mutant	Segregation ratio(wild: mutants)
vigorous×Em'A'	100	43	67	1:1.56
ropy×Em'A'	110	49	61	1:1.25
buff×Em'A'	96	45	51	1:1.13
albino×Em'A'	110	50	60	1:1.2
fluffy×Em'A'	100	51	49	1:0.96
conidial band×Em'A'	120	53	63	1:1.26
conidial lump×Em'A'	100	44	56	1:1.27
plug×Em'A'	120	59	61	1:1.03

PREPARATION OF EXTRACTS OF SOME MUTANTS FOR T HE STUDY OF LINKAGE

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

Table-22

Result of the cross of mutants and markers

Name of the mutant	Name of the cross	Perithesia formed in days	Days taken for spore shedding	Size and No. of perithesia	Linkage group of the marker	Fertility with the marker
vigorous	1590×leu-3A	10	17	Medium,,many	I	Fertile with leu-3A
	1590×arg-5A	10	17	medium,many	II	Fertile with Arg-5A
	1590×trp-1A	7	21	Medium, many	III	Fertile with trp-1A
	1590×trp-4A	7	23	Medium& some	IV	Fertile with trp-4A
	1590×lys-1A	7	19	Medium& some	V	Fertile with lys-1A
	1590×ad-1A	10	19	medium& few	VI	Fertile with ad-1A
	1590×arg-10A	10	28	Medium & few	VII	Fertile with arg-10A

ropy	517×leu-3A	7	15	Small & some	I	Fertile with leu-3A
	517×arg-5A	7	15	Large &many	II	Fertile with Arg-5A
	517×trp-1A	9	15	Large &many	III	Fertile with trp-1A
	517×trp-4A	9	17	Medium &many	IV	Fertile with trp-4A
	517×lys-1A	8	18	Large &few	V	Fertile with lys-1A
	517×ad-1A	8		Medium & few	VI	Not fertile with ad-1A
	517×arg-10A	8	19	Medium & few	VII	Fertile with arg-10A
buff	173×leu-3A	7	17	Large &many	I	Fertile with leu-3A
	173×arg-5A	7	17	Small & many	II	Fertile with Arg-5A
	173×trp-1A	7	21	Large &many	III	Fertile with trp-1A
	173×trp-4A	9	23	Medium & some	IV	Fertile with trp-4A
	173×lys-1A	9	15	Small & 10-12	V	Fertile with lys-1A
	173×ad-1A	15	15	Medium & some	VI	Fertile with ad-1A
	173×arg-10A	15	15	Medium & many	VII	Fertile with arg-10A

albino	1223×leu-3A	15		Large & 2-3	I	Not fertile with leu-3A
	1223×arg-5A	15		Large & 9-10	II	Not fertile with arg 5A
	1223× trp-1A	10	23	Large & few	III	Not fertile with trp-1A
	1223×trp-4A	10	23	Large & few	IV	Fertile with trp-4A
	1223×lys-1A	9		Small & few	V	Fertile with lys-1A
	1223×ad-1A	9	21	Large & 2-3	VI	Not fertile with ad-1A
	1223×arg-10A	9	21	Large & few	VII	Fertile with arg-10A
fluffy	55×leu-3A	7	17	Small &many	I	Fertile with leu-3A
	55×arg-5A	7	17	Small & many	II	Fertile with Arg-5A
	55× trp-1A	9	21	Medium & some	III	Fertile with trp-1A
	55×trp-4A	8	21	Medium & some	IV	Fertile with trp-4A
	55×lys-1A	8	21	Large &many	V	Fertile with lys-1A
	55×ad-1A	10	23	Small &some	VI	Fertile with ad-1A
	55×arg-10A	9	23	Small &many	VII	Fertile with leu-3A
conidial band	E1 753×leu-3A	7	21	Small &many	I	Fertile with leu-3A

	E1 753×Arg-5A	7	28	Medium & few	II	Fertile with Arg-5A
	E1 753× trp-1A	8	23	Small &many	III	Fertile with trp-1A
	E1 753×trp-4A	8	27	Large &3	IV	Fertile with trp-4A
	E1 753×lys-1A	8	17	Medium & many	V	Fertile with lys-1A
	E1 753×ad-1A	7	18	Medium & few	VI	Fertile with ad-1A
	E1 753×arg-10A	7	20	Small & few	VII	Fertile with arg-10A
conidial lump	A1 1557×leu-3A	7	17	Small &many	I	Fertile with leu-3A
	A1 1557×Arg-5A	8	21	Small &many	II	Fertile with Arg-5A
	A1 1557× trp-1A	8	21	Medium & some	III	Fertile with trp-1A
	A1 1557×trp-4A	7	15	Large &many	IV	Fertile with trp-4A
	A1 1557×lys-1A	7	15	Small &some	V	Fertile with lys-1A
	A1 1557×ad-1A	7	17	Small &some	VI	Fertile with ad-1A
	A1 1557×leu-3A	7	18	Small & few	VII	Fertile with arg-10A

plug	D4 100×leu-3A	7	20	Small &many	I	Fertile with leu-3A
	D4 100×Arg-5A	7	21	Small &many	II	Fertile with Arg-5A
	D4 100× trp-1A	8	21	Medium & some	III	Fertile with trp-1A
	D4 100×trp-4A	8	17	Large &many	IV	Fertile with trp-4A
	D4 100×lys-1A	9	16	Small &some	V	Fertile with lys-1A
	D4 100×ad-1A	9	16	Small &some	VI	Fertile with ad-1A
	D4 100×leu-3A	9	18	Small & few	VII	Fertile with arg-10A

STUDY OF LINKAGE OF SOME SELECTED MUTANTS WITH THE MARKERS OF DIFFERENT LINKAGE GROUP.

Spores from the crosses were spread on SM supplemented plate. They were kept at 58C foe 50 minutes and then incubated at 25C for 12-16 hours. The germinatin growing spores were isolated randomly and inoculated into small tubes containing VM supplemented with necessary aminoacid. The culture were incubated at 25C for 4-6 days they were tested on SM and Sm supplemented plates. Four types of progeny (i) Mutant (ii) Marker (iii) Marker+ Mutant and (iv) Wild were expected. The linkage was studied by analyzing the percentage of recombiant and parental types.

Fig: 13. A fertile cross showing huge mature perithecia

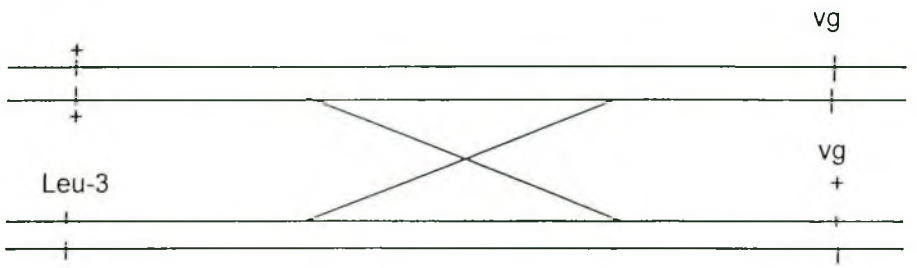
Table-23

Linkage of vigorous (1590) mutants:

Mutant used	Marker used	Linkage group	Cross	Total isolates	Progenies	% of each progeny	Inference on linkage
vigorous (1590)	leu3(R156A)	I	leu-3A×vg(1590)	106	wild=19 leu-3A=36 vg=37 vg+leu=14	wild=17.92 leu-3A=33.96 vg=34.96 vg+leu=13.20	Linked with leu-3 of linkage group-1
vigorous (1590)	Trp-1(A106)	III	Trp-1A×vg	96	wild=22 trp-1=23 vg=24 vg+trp=27	wild=22.91 trp-1=23.95 vg=25 vg+trp=28.15	Not linked with linkage group-3
vigorous (1590)	Trp-4(Y2198)	IV	Trp-4A×vg	98	wild=25 trp-4A=21 vg=24 vg+leu=28	wild=25.5 trp-4A=21.42 vg=24.48 vg+leu=28.57	Not linked with linkage group-4
vigorous (1590)	Ad-1(3251)	VI	Ad-1A×vg	100	wild=29 ad-1A=21 vg=24 vg+Ad=26	wild=29 ad-1A=21 vg=24 vg+ad=26	Not linked with linkage group-3
vigorous (1590)	Arg10A(B217)	VII	Arg-10A×vg	103	wild=26 arg-10A=24 vg=25 vg+Arg=28	wild=25.24 arg-10A=23.30 vg=24.27 vg+Arg=27.18	Not linked with linkage group-3

9.1.8 Determination of Genetic Map of vigorous (U-517)mutant.

Crossing name :Vg(U-517) X leu-3(R156)A



$$\text{Distance} = \frac{R_1+R_2}{\text{Total progenies}} \times 100(\text{centimorgan})$$

Here
R_1+R_2=Recombinants

$$= \frac{19+14}{106} \times 100(\text{centimorgan})$$

= 31.13 centimorgan

31.13

Fig- :Genetic Map of vg (U-517) mutant.

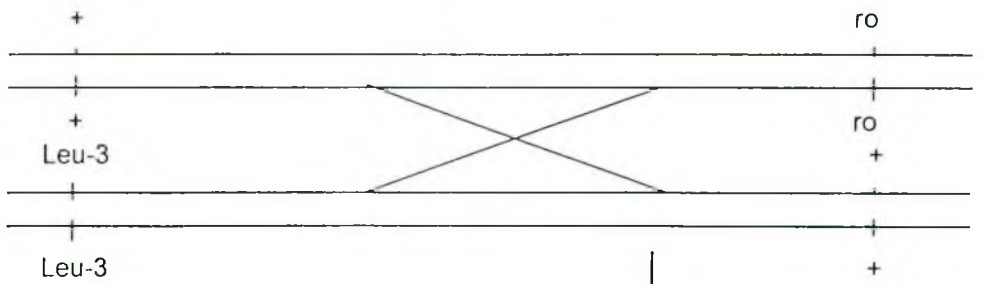
Table-24

Linkage of *ropy* (517) mutants:

Mutant used	Marker used	Linkage group	Cross	Total isolates	Progenies	% of each progeny	Inference on linkage
ropy (517)	leu3(R156A)	I	leu-3A×ropy	95	wild=16 leu-3A=31 ry=31 ry+leu=17	wild=16.84 leu-3A=32.63 ry=32.63 ry+leu=17.89	Linked with leu-3 of linkage group-I
ropy (517)	trp-1(A106)	III	trp-1A× ropy	91	wild=25 trp-1=41 ry=8 ry+trp=17	wild=24.47 trp-1=50.61 ry=9.87 ry+trp=20.9	Not linked with linkage group-III
ropy (517	trp-4(Y2198)	IV	trp-4A× ropy	96	wild=14 trp-4A=25 ry=12 ry+trp=45	wild=14.58 trp-4A=26.04 ry=12.5 ry+trp =46.87	Not linked with linkage group-IV
ropy (517)	ad-1(3254)	VI	ad-1A× ropy	94	wild=23 ad-1A=24 ry=21 ry+Ad=26	wild=24.46 ad-1A=25.53 ry=22.34 ry+Ad=27.65	Not linked with linkage group-VI
ropy (517)	arg10A(B217)	VII	arg-10A× Ropy	80	wild=24 arg-10A=15 ry=26 ry+Arg=15	wild=30 arg-10A=18.75 rg=32.5 vg+arg=18.75	Not linked with linkage group-VII

9.1.8 Determination of Genetic Map of ropy (U-517)mutant.

Crossing name :ropy(U-517) X leu-3(R156)A



$$\text{Distance} = \frac{R_1+R_2}{\text{Total progenies}} \times 100(\text{centimorgan})$$

Here $R_1+R_2=Re$

$$= \frac{16+17}{95} \times 100(\text{centimorgan})$$

= 34.73 centimorgan

34.73

Fig- Genetic Map of ropy (U-517) mutant.

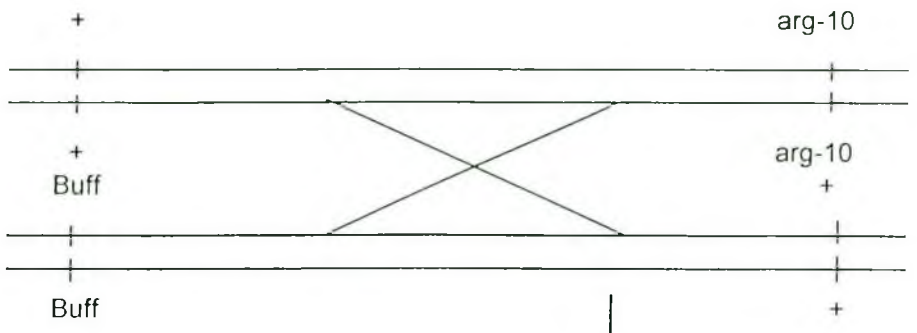
Table-25

Linkage of Buff (173) mutants:

Mutant used	Marker used	Linkage group	Cross	Total isolates	Progenies	% of each progeny	Inference on linkage
buff (173)	Leu3(R156A)	I	Leu-3A× *buff*	120	wild=29 Leu-1=33 *buff* =27 *buff* + trp=31	wild=24.17 Leu-1=27.5 *buff* =24 *buff* +trp=25.83	Not linked with leu-3 of linkage group-1
buff (173)	Trp-1(A106)	III	Trp-1A× *buff*	120	Wild=36 trp-1=38 *buff* =20 *buff* +trp=26	Wild=30 trp-1=31.67 *buff* =16.68 *buff* +trp=21.68	Not linked with linkage group-III
buff (173)	Trp-4(Y2198)	IV	Trp-4A× *buff*	100	Wild=24 trp-4A=21 *buff* =30 *buff* +leu=25	Wild=24 trp-4A=21 *buff* =30 *buff* =25	Not linked with linkage group-IV
buff (173)	Ad-1(3254)	VI	Ad-1A× *buff*	100	Wild=27 Ad-1A=28 *buff* =23 *buff* +Ad=22	Wild=27 Ad-1A=21 *buff* =28 *buff* =23 *buff* +Ad=22	Not linked with linkage group-VI
buff(173)	Arg10A(B217)	VII	Arg-10A× buff	100	Wild=16 Arg-10A=35 *buff* =34 *buff* +Arg=15	Wild=16 Arg-10A=35 buff =34 *buff* +Arg=15	Linked with linkage group-VII

9.1.4 Determination of Genetic Map of Buff(U-173)mutant

Crossing name :Buff(U-173) X arg-10A



$$\text{Distance} = \frac{R_1+R_2}{\text{Total progenies}} \times 100(\text{centimorgan})$$

$$= \frac{15+16}{100} \times 100(\text{centimorgan})$$

$$= 31\text{centimorgan}$$

Here
R_1+R_2=Recombinants

Fig-22:Genetic Map of Buff (U-173) mutant.

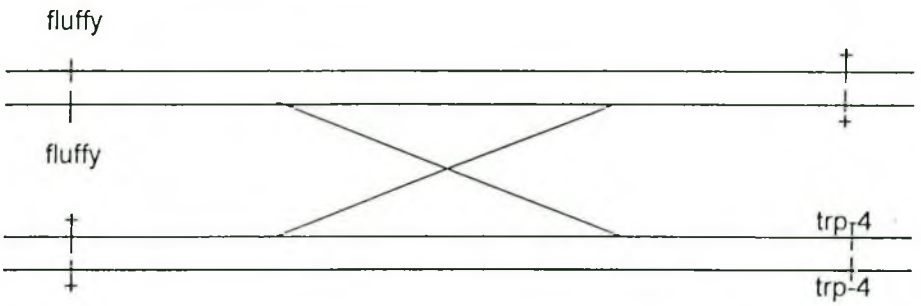
Table-26

Linkage of *fluffy* (55) mutants:

Mutant used	Marker used	Linkage group	Cross	Total isolates	Progenies	% of each progeny	Inference on linkage
fluffy (55)	Leu3(R156A)	I	Leu-3A×Ffy	130	Wild=33 Leu-3A=32 Ffy=34 Ffy +leu=31	Wild=25.38 Leu-3A=24.61 Ffy =26.15 Ffy +leu=23.84	Not linked with leu-3 of linkage group-1
fluffy (55)	Trp-1(A106)	III	Trp-1A× Ffy	120	Wild=29 trp-1=35 *fl* =27 *fl* +trp=29	Wild=24.17 trp-1=29.17 Ffy =22.5 Ffy +trp=24.17	Not linked with linkage group-III
fluffy (55)	Trp-4(Y2198)	IV	Trp-4A× Ffy	120	Wild=16 trp-4A=44 *C* =41 *fl* +leu=19	Wild=13.33 trp-4A=36.68 Ffy =34.17 Ffy +leu=15.83	Linked with linkage group-IV
fluffy (55)	Ad-1(3254)	VI	Ad-1A× Ffy	100	Wild=24 Ad-1A=24 *fl* =31 *fl* +Ad=21	Wild=24 Ad-1A=24 *fl* =31 *fl* +Ad=21	Not linked with linkage group-VI
fluffy (55)	Arg10A(B217)	VII	Arg-10A× Ffy	100	Wild=29 Arg-10A=21 Ffy =28 Ffy +Arg=22	Wild=29 Arg-10A=21 Ffy =28 Ffy +Arg=22	Not linked with linkage groupVII

9.1.4 Determination of Genetic Map of fluffy(U-55)

Crossing name :fluffy(U-55)Xtrp-4



$$\text{Distance} = \frac{R_1+R_2}{120} \times 100 = \frac{19+16}{120} \times 100$$

= 29.17Centimorgan

Fig-30:Genetic Map of fluffy (U-55)

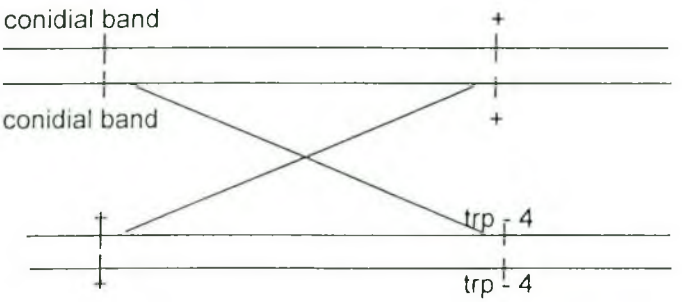
Table-27

Linkage of Conidial Band (E1753) mutant:

Mutant used	Marker used	Linkage group	Cross	Total isolates	Progenies	% of each progeny	Inference on linkage
conidial band (E1753)	leu3(R156A)	I	leu-3A×C.b.	100	Wild23 Leu-3A=20 C.b.=33 C.b.+leu=24	Wild=23 Leu-3A=20 C.b.=33 C.b.+leu=24	Not linked with leu-3 of linkage group-1
conidial band (E1753)	trp-1(A106)	III	trp-1A× c.b.	100	Wild=26trp-1=21 C.b.=30 C.b.+trp=23	Wild=26 trp-1=21 C.b.=30 C.b.+trp=23	Not linked with linkage group-III
conidial band (E1753)	trp-4(Y2198)	IV	trp-4A× c.b.	120	Wild=9 trp-4A=50 C.b.=47 C.b.+leu=14	Wild=7.5 trp-4A=41.67 C.b.=39.17 C.b.+leu=11.67	linked with linkage group IV
conidial band (E1753)	ad-1(3254)	VI	ad-1A× C.b.	88	Wild=25 Ad-1A=24 C.b.=24 C.b.+Ad=15	Wild=28.40 Ad-1A=27.27 C.b.=27.27 C.b.+Ad=17.04	Not linked with linkage groupVI
conidial band (E1753)	arg10A(B217)	VII	arg-10A× C.b.	130	Wild=35 Arg-10A=32 C.b.=33 C.b.+Arg=30	Wild=26.92 Arg-10A=24.61 C.b.=25.38 C.b.+Arg=23.08	Not linked with linkage groupVII

Determination of Genetic Map of conidial band($E_1$753)

Crossing name :Conidial band($E_1$753)Xtrp-4



$$\text{Distance} = \frac{R_1+R_2}{120} \times 100 = \frac{14+9}{120} \times 100$$

= 19.17 Centimorgan

Fig-31:Genetic Map of conidial band($E_1$753)

Table-34
Distances of the mutant with the marker

Mutant used	Marker used	Linkage group of the marker	Distance of the mutant with marker
vigorous (U517)	lue-3 (R156)A	I	leu-3 — 31.13 — Vg
ropy (U-517)	lue-3 (R156)A	I	leu-3 — 34.73 — ro
buff (U-173)	arg-10	VII	Buff — 31 — arg-10
fluffy 55	Trp4A	IV	Trp4 — 29.17 — F1
conidial band (E.753)	Trp4A	IV	Con band — 19.17 — trp4

Discussion

Stadler *et. al.(*1993) reported that spontaneous mutation in *Neurospora crassa* is very rare and frequent in nature. The author checked if there was any spontaneous mutation in *Neurospora crassa.* In this investigation conidia from fresh culture of Ema 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 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 Micribial Laboratory, Department of botany, University of Dhaka also did not obtained any spontaneous mutation (Mozmader et.al. 1997).

The types of mutants obtained following treatment with Rhizome extract of *Asparagus recemosus* Linn., leaf extract of *Datura metel* L., *Eclipta prostrata* Less. and UV are slightly different from that found earlier with formaldehyde vapour(Mozmader *et. al.* 1995). fomaldehyde solution(Mozmader *et. al.* 1997) and phenol solution (Mozmader *et. al.* 1986).checked growth, vigorous growth, band and cauliflower type were obtained when formaldehyde vapour was used and in case of formaldehyde solution vigorous growth, check growth, plug, band and yellow were obtained. With phenol vigorous, mat, *checked,fluffy* and band types were obtained.

This discrepancies could be due to the difference in mutagenic specificity of formaldehyde vapour, formaldehyde solution, phenol, ethyle methane sulphonate(EMS), rhizome extract of *Asparagus recemosus* Wild, leaf extract of Detura metel L. and UV irradiation.

Raper *et al* (1965); Fedorov, SN. Zaretskaia, AN. (1978); Greer, WL.Wellman,AM.(1980); Nieto, DJ.Woods, RA. (1983); and Miasniankina *et al* (1983) also discovered mutagenic activity of ethyle methane sulphonate (EMS) and showed that it mutagenized stain of *Schizophillum commune* , *Rhizobium meliloti, Nurospora*

crassa,Saccharomyces cerevisiae, Drosophilla melanogaster and produced mutants.

The variants with changed morphology were classified into 9 groups: *check, ropy, conidial band, vigorous. albino, buff, fluffy ,plug* and *conidial lump.*

Fresh conidia of the selected representatives *check,conidial band , buff, fluffy, plug , albino* and were included in VM plates for conidial germination and observations were recorded up to 8 hours.

It is found that there was no indication of germination conidia or mycelia growth up to 2 hours in the type Em'a' or any of the mutants. After 4 hours growth was initiated in wild type Em'a' as well as in mutants namely fluffy band ,buff, plug, conidial band, and vigorous germinated after 8 hours.

Fresh conidia were inoculated in VM plates for determination of radial growth of the wild type and some selected mutants *fluffy, check, ropy, conidial band, vigorous, buff, fluffy, plug, conidial lump* and *albino.* Observation after 6 hours up to 48 hours were recorded.

After 36 hours wild type Em'a' grew up to 4.05cm whereas mutant *plug* grew up to 5.0cm only.

Fresh conidia were also inoculated in race tube (Ryan 943) for the study of linear growth of the mycelia of the mutants and observations were taken after 6 hours up to 96 hours. (Table)

When linear growth were undertaken interesting results were found as shown in table. The mutants differed in their liner growth from the liner growth found with Em'a'.Thus plug gave about 1.09 times as much as the growth of Em'a.The growth of plug was significantly higher (4.00 cm) and the growth of buff was lower (24.25 cm).

Fresh conidia were inoculated further in liquid VM media in conical flasks for obtaining Biomass of the wild type Em'a' and some selected mutants, ropy, conidial band, Buff, fluffy, plug, albino ,vigorous, conidial lump. Mycelia were harvested by filtering after 72 hours

washed with distilled water and dried in the oven at 18°C and weighted

After 72 hours willed type Em'a' gave a mass of 0.284 g. the maximum mass (0.344 g) was obtained incase of plug and the lowest (0.113 g) was obtained incase of mat.Suggests that the morphological changes with mutants might be the result of a change in molecular gene.

Representative were selected from each group and were crossed with Em'a' and Em'A' separately. All process of selected mutants were fertile and the following are less fertile fluffy, albino. However all of them formed enough perethecia and shed spores when sugar 2% and agar 2% were used in the Westergaard's crossing medium. Perthecia formed in almost all process with Em'A' which showed that mating types were 'a' and during induction of mutants their mating were not changed along with the morphological change.

The discrepancy in performance of the different crosses can be explained by assumption.

That the mutants are not of the same vigor vis a vis their vegetative growth. Some mutants are more vigorous in their growth than others.

Ascospores from the fertile process were spread on SM plates after 12 hours single growing spores were randomly isolated into VM tubes and inoculated at 25°C for growth. After 4-5days they were analyzed for mutant type and wild type morphology.

Analysis of the progenies from the fertile crosses of the mutants with wild type indicates that each mutant segregated in the ratio 1:1. This finding suggests that the morphological changes with mutants might be the result of change in nuclear gene. The selected mutants were also crossed with the representatives of different linkage groups. It was found that mutant vigorous was found to be linked with leu-3(R156)A of linkage group-I and gave a distance of 31.13 centimorgan.

Mutants conidial band and fluffy were found to be linked with trp-4A of linkage group-IV and gave adistance of 19.17 and 29.17 centimorgan respectively. Mutants ropy and buff were found to be linked with leu-3A(R156)A and arg-10A of linkage group-I and VII both gave a distance 34.73 and 31 centimorgan respectively.

About 4000 conidia from each culture of Em'a' were spread from SM plates. Distincly grown single conidial colonics were isolated individually in VM tubes. After 4-5 days when sufficient conidia were formed individual cultures were analyzed for the presence of any morphological variations. None of the isolates were found to show morphological variations and all of them resembled the parental stock.

This indicates that the morphological variants obtained from the treatment with rhizome extract of *Asparagus recemosus* Linn.,leaf extract of *Datura metel* Linn., *Eclipta prostrata* Linn. and UV might be due to chemical mutagenic and not due spontaneous mutation in Em'a' stock.

It appears from that plug germinates after 5 hours, grew up to 40.0cm in 96 hours and gave a growth of 1.09 times more biomass than wild type in 72 hours, whereas mat germinated after 8 hours and gave linear growth of 10.05 cm during the same period of time and gave a biomass which was 0.27 times less than the wild type Em'a'. . The time taken for germination of conidia also seems to be directly related to their linear growth of mycelia and weight of biomass.

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