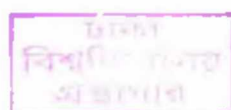


**INDUCTION OF MUTATION WITH
BOTANO-CHEMICALS
IN
Neurospora crassa, CLASSIFICATION,
CHARACTERIZATION AND GENETIC
STUDIES OF INDUCED MUTANTS.**

448581



MASTER OF PHILOSOPHY



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

Md. Siddiqur Rahman
Reg. No.- 228, Roll No : 01
Session : 2002-2003

October 2008

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A THESIS

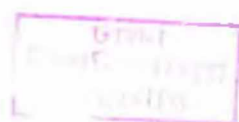
**SUBMITTED IN PARTIAL FULFILMENT FOR THE
DEGREE OF MASTER OF PHILOSOPHY
IN BOTANY, FACULTY OF BIOLOGICAL SCIENCES,
UNIVERSITY OF DHAKA.**

448581

BY

MD. SIDDIQUR RAHMAN

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

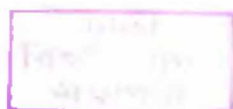


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University of Dhaka,
Dhaka-1000, Bangladesh.**

October 2008

**DEDICATED
TO
MY MOTHER
MRS. ANUWARA BEGUM**

448581



CERTIFICATE

I hereby certify that the work reported here was carried out by Md. Siddiquir Rahman under my guidance and Supervision. It is further certified that the work presented here is original and suitable for submission as an M. Phil Thesis.

Tahsina Rahim
8.10.08

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Professor:
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University of Dhaka.

DECLARATION

I hereby declare that this Thesis entitled “Induction of mutation with Botano-chemicals in *Neurospora crassa*, Classification, Characterization and genetic studies of induced mutants”, has been composed by myself and all the work presented here in are my own. I further declare that this work has not been submitted anywhere for any academic degree.

October-2008

Md. Siddiquir Rahman
Md. Siddiquir Rahman
Reg. No.- 228, Roll No : 01
Session : 2002-2003

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LIST OF ABBREVIATIONS

%	=	Percent
<i>al</i>	=	<i>albino</i>
<i>arg</i>	=	Arginine
<i>arg-10</i>	=	Arginine -10 auxotroph
<i>arg-5</i>	=	Arginine -5 auxotroph
<i>buf</i>	=	<i>buff</i>
<i>C</i>	=	<i>Coccinea cordifolia</i>
<i>C_p</i>	=	(100%) Pure crude extract of <i>Coccinea cordifolia</i>
<i>C₁</i>	=	(50%) crude extract of <i>Coccinea cordifolia</i>
<i>C₂</i>	=	(33.33%) crude extract of <i>Coccinea cordifolia</i>
<i>C₃</i>	=	(25%) crude extract of <i>Coccinea cordifolia</i>
<i>C₄</i>	=	(20%) crude extract of <i>Coccinea cordifolia</i>
<i>C₀</i>	=	No crude extract only VM media
<i>ch</i>	=	<i>check</i>
<i>clf</i>	=	<i>cauliflower</i>
<i>crs.</i>	=	<i>Crescent</i>
<i>cm</i>	=	Centimeter
<i>con.</i>	=	<i>conidial band</i>
<i>band</i>		
<i>dir</i>	=	<i>dirty</i>
<i>Em</i>	=	Emerson stock
<i>Ema</i>	=	Emerson stock mating type 'a'
<i>EmA</i>	=	Emerson stock mating type 'A'
<i>fl.</i>	=	<i>fluffy</i>
<i>fl. band</i>	=	<i>fluffy band</i>
<i>g.</i>	=	Gram
<i>K</i>	=	Kamranga
<i>K_p</i>	=	(100%) Pure crude extract of Kamranga

K ₁	=	(50%) crude extract of Kamranga
K ₂	=	(33.33%) crude extract of Kamranga
K ₃	=	(25%) crude extract of Kamranga
K ₄	=	(20%) crude extract of Kamranga
K ₀	=	No crude extract only VM media
leu -1	=	Leucine -1 auxotroph
Leu -3	=	Leucine -3 auxotroph
Luc	=	Leucine
mg	=	Milligram
ml	=	Milliliter
O _p	=	(100%) Pure crude extract
O ₁	=	(50%) crude extract
O ₂	=	(33.33%) crude extract
O ₃	=	(25%) crude extract
O ₄	=	(20%) crude extract
O ₀	=	No crude extract only VM media
or	=	<i>orange</i>
ro	=	<i>ropy</i>
S	=	Shetadron
S _p	=	(100%) Pure crude extract of Shetadron
S ₁	=	(50%) crude extract of Shetadron
S ₂	=	(33.33%) crude extract of Shetadron
S ₃	=	(25%) crude extract of Shetadron
S ₄	=	(20%) crude extract of Shetadron
S ₀	=	No crude extract only VM media
SM	=	Sorbose minimal medium
SM ⁺	=	Supplemented Sorbose minimal medium
T	=	Tulshi
T _p	=	(100%) Pure crude extract of Tulshi
T ₁	=	(50%) crude extract of Tulshi

T ₂	=	(33.33%) crude extract of Tulshi
T ₃	=	(25%) crude extract of Tulshi
T ₄	=	(20%) crude extract of Tulshi
T ₀	=	No crude extract only VM media
trp	=	Tryptophan
trp -4	=	Tryptophan -4 auxotroph
trp-2	=	Tryptophan -2 auxotroph
trp-5	=	Tryptophan-5 auxotroph.
UV	=	Ultra Violet
Vg.	=	<i>vigorous</i>
VM	=	Vogel's minimal medium
VM+	=	Supplemented vogel's minimal medium
WCM	=	Westergard's crossing medium
<i>y.fl. band</i>	=	<i>yellow fluffy band</i>

ABSTRACT

Wild type strain Em'a' (5297) of *Neurospora crassa* was analyzed for spontaneous mutation. But it was found that there was no spontaneous mutation in it.

It has been found that leaf extracts of *Ocimum sanctum* Linn. controlled the radial growth of wild type Em'a' of *Neurospora crassa*. The result also showed that 100% concentration of crude extract was more effective than 50% or 25% concentration. 100% crude extract was chosen for treating the conidia of Em'a' for induction of mutation and nine groups of mutations were found. These are *check*, *ropy*, *albino*, *vigorous*, *conidial bad*, *fluffy*, *mat*, *cauliflower* and *dirty*.

It has been found that the fruit extract of *Averrhoa carambola* Linn. controlled radial growth of *Neurospora crassa*. The result also showed that 100% concentration of crude extract was more effective than 50% or 25%. 100% crude extract was chosen for treating the conidia of Em'a' for induction of mutation and twelve groups of mutants were found. These are *ropy*, *albino*, *plug*, *vigorous*, *conidial band*, *fluffy*, *fluffy band*, *check*, *mat*, *yellow fluffy*, *cauliflower* and *orange*.

Leaf extract of *Leucas lavandulaefolia* Rees. possesses antifungal properties and it reduced the radial growth of *Neurospora crassa* Ema. Conidia of Ema were also treated with leaf extract of *Leucas lavandulaefolia* Rees. and 11 groups of mutants were found: *ropy*, *plug*, *vigorous*, *albino*, *conidial band*, *fluffy*, *check*, *mat*, *cauliflower*, *fluffy band* and *dirty*.

Leaf extract of *Coccinea cordifolia* Linn. possesses antifungal properties and it reduced the radial growth of *Neurospora crassa* Ema. Conidia of Ema were also treated with the leaf extract of *Coccinea cordifolia* Linn. and 10 groups of mutants were found: *ropy*, *albino*, *plug*, *fluffy*, *mat*, *conidial band*, *cauliflower*, *check*, *fluffy band* and *vigorous*.

UV induction resulted 13 groups of morphological mutants: *ropy*, *albino*, *mat*, *plug*, *vigorous*, *fluffy*, *mat conidial band*, *crescent*, *orange*, *cauliflower*, *fluffy band* and *dirty*.

Morphological mutants induced with leaf extract of *Ocimum sanctum* Linn., fruit extract of *Averrhoa carambola* Linn., leaf extract of *Leucas lavadulaefolia* Rees., leaf extract of *Coccinea cordifolia* Linn. and UV irradiation were selected for detailed study and analysis. The mutants showed variation with Ema in their morphology, weight of the mycelia, Linear growth rate, initiation of conidial germination, fertility, mating type and segregating ratio were also checked.

All the mutants have been crossed with Em and the segregation of wild : mutant is found to be in the ratio 1 : 1. Therefore, these morphological mutants are nuclear in nature. Heterocaryosis of similar morphological mutants were done to see whether similar morphological mutants show any complementation. The result were negative.

Linkage study of *vg.* (K₄-470), *con band* (K₂-299), *fl.* (C₃-366), *ropy* (K₄-409), *mat* (S₄-411), and *ch*(T₃-317) and *fl band* (C₄-452) were undertaken by crossing them with marker representatives of all the seven linkage groups. *vg*(K₄-470), *fl*(C₃-366) and *ropy* (K₄-409) are linked with *leu-3* of linkage group-I; *con-band* (K₂-299) and *mat* (S₄-411) are linked with *trp-I* of linkage group – III; *clff*(U-256) and *ch*.(T₃-317) are linked with *trp-4* of linkage group-IV.

INTRODUCTION

INTRODUCTION

Neurospora crassa is an excellent tool for genetical study. *Neurospora crassa* has proved to be an important genetical material for its profuse growth in synthetic media, haploid nature, linear arrangement of its ascospores and as a result it can be used for any classical as well as molecular investigations. It is a well known pink mold. This filamentous fungus belongs to the class- Ascomycetes, sub class- Pyrenomycetadae, order- Xylariales, family- Sordariaceae. It is non pathogenic and can be easily grown on a suitable mixture of mineral salt in water and a carbon source (Sucrose) and the vitamin, biotin (Beadle and Tatum 1941). Anonymous, 1966) Both growth on race tube (Ryan *et al* 1943) and dry weight of mycelia (Beadle and Tatum, 1941) are useful or accurate measurement of growth, the former measuring rate and the latter terminal growth mass.

The vegetative phase consists of a mycelium of branching hyphae and only the zygote is diploid.

Neurospora crassa is octosporous, hermaphroditic, heterothallic and as a result there are two mating types called 'A' and 'a'.

It has distinct male and female sexual structure. It produces great masses of brilliant, orange red asexual spores.

This fungus has many advantages in the study of what genes do. Its genes can conveniently be changed artificially and the part they play in the chemical alteration and metabolism of cells can be analysed with considerable precision (George, 1984.).

It can be multiplied a million fold in a few days without any genetic change (Pandit and Maheswari, 1993).

The geneticists are interested to know extensively about genetics. They are trying to solve the genetic riddle by studying the organization of genes the tiny transmitters of hereditary traits. Genes are located on the chromosome as small metabolically differentiated regions. Each gene can effect the character of

a cell in a highly specific way. The most favourable objects for use in studying what a gene is and how it functions are the haploid microorganisms like bacteria and their viruses, the bacteriophages and fungi. They have the advantage of having very short life cycle and ease of growth under controlled laboratory conditions (Watson, 1970).

De- oxyribose nucleic acid or DNA is the active principal of genes of all living organisms, which creates and brings life gradually into bloom through the production of specific proteins at specific times. Sometimes this gene changes or mutates resulting in a change of the phenotype of the organism. Benzer (1957) suggested new terminology as to be certain of wide spread adoption. The accepted concept of gene a particular hereditary determiner, a unit of inheritance located in a fixed location in the chromosomes (Gardner, 1972).

Genetic analysis begun by Morgan in 1909 and by the subsequent studies arose the concept of gene as a unit of recombination (Sturtevant, 1913) when in 1927 at the 5th International congress of genetics in Barlin, Muller from united states first reported the production of mutations in *Drosophila* by X-rays, he opened a new chapter in the history of genetics (Auerbach, 1962). Gene, the substance of which is DNA or RNA or Nucleoprotein complex is very important as it controls all the physical and bio-chemical activities of an organism whether it is a virus or a bacterium, an alga, a fungus, a bryophyta, a pteridophyta or an angiosperm. We cannot see a gene with our own naked eyes or with the help of the most powerful microscope. But, we can know about a particular gene only when it mutates. Mutation is, therefore, a very important phenomenon in genetics.

The most technically advantageous method of studying the organization and mode of action of gene or the genic inheritance in any organism is achieved by inducing artificial mutation in the organism at different loci. Without mutation the loci could never be identified; nor their functions studied.

A particular gene is responsible for one or more defined biochemical reactions. So if there is any change in the body of one gene or more genes, a new

phenotypic character or character will follow in the organisms concerned. The change of gene in the body of the organisms is called mutation.

By application of the artificial mutation many mutants can be obtained which fail to grow unless some specific substances such as amino acid or vitamin are added to the culture medium. The actual mutation is supposed to take place in the level of DNA structure. Mutation are changes in the sequence of base pair. If there occurs any change in the sequence of bases in DNA molecules it may change the whole course or a part of metabolic activities of the living organism and this phenomenon is known as mutation.

The genetics and physiology of *Neurospora crassa* have been studied intensively. The study on the suitable mutagens for this organism and the study on molecular mechanism of mutation have been the subjects of the investigation during the past years (Beadle and Tatum, 1945; Hollaender *et al* 1945; Bonner, 1946, Ahmad and catcheside, 1960; Flavel and Fincham, 1968, Schroeder, 1970; Brockman and De Serres 1964; Ahmad *et al*, 1972; Santosa and kuwana, 1992; Stephens *et al* 1993 and Vellanji *et al*, 1994).

Now there is increasing use of mutations specifically induced by external agents such as ionizing radiation, Ultra-violet light and certain specific chemicals. These agents collectively called mutagens, greatly increase the rate at which geneticists can isolate mutant genes. For many years the various forms of radiation were the most powerful mutagens known.

Mutagen act quite indiscriminately. No presently known mutagen increases the probability of mutating a given gene without also increasing the probability of mutating all other genes. Until recently the mechanisms of mutagenesis were completely unclear. Alterburg has found that UV light is very effective in producing mutation in *Drosophila*. UV light is specially effective as a mutagenic agent in microorganism.

In *Neurospora crassa* most mutant character have involved either biochemical deficiencies or morphological differences in which mutant phenotypes were distinguishable in haploid strain. In the Genetical Research

Laboratory of Dhaka University Ahmad and Mozmader in 1965 irradiated the conidia of *Neurospora crassa* strain Emerson 'a' (5297) with uv light and isolated a number of biochemical mutants along with the morphological mutants.

Mutagens are physical or chemical agents such as radiation, heat or alkylating or deaminating agents, which raise the frequency of mutation, greatly above the spontaneous background level. Chemical mutagens have the ability to penetrate cells and to alter the DNA within cell, (Fishbein *et al* 1970). Chemicals that have mutagenic properties include peroxides, formaldehyde (Gardner, 1981), permanganate, urethane, nitrous acid, Ethyl methane sulphonate (EMS), Musturd gas (Alterberg; 1957) and chloramphenical.

In 1939, Steinberg first reported the production of mutation by chemical treatment of cell. Study of Kolmarks and westergaard have been shown that Bromoethyl methane sulphonate when used on *Neurospora crassa* causes mutation. Admirable accounts both of genetical and much of biochemical work is given by Catcheside (1949) and Horowitz (1973.) perhaps the most extensive and systemic surveys of the action of biochemical mutagens on fungi have been made by Westergaard on *Neurospora crassa*. (Fincham and Day 1965). Since the discovery of physical mutagens by Mullar, H.J. (1916) and chemical mutagens by Thomb and Steinberg (1939), Auerbech and Robson (1946) a large number of mutagens have been discovery and the list is increasing with the passing of years.

Plant extracts play an important role to check the growth of various fungi. Recently some workers Tripathi *et al.* (1978), Lacandula (1993), Solsoloy *et al.* (1993), Kale *et al.* (1995) and Garcia and Padilla (1996) all over the world have been studying the botano-chemicals for such purpose.

The author tested the mutagenic effect of leaf extract of *Ocimum sanctum* Linn. (Tulshi), *Leucas lavandulaefolia* Rees. (Shetadron), *Coccinea cordifolia* Linn. (Telakucha) and fruit extract of *Averrhoa carambola* Linn. (kamranga) on *Neurospora crassa*.

Ocimum sanctum Linn. leaves contain phenols, aldehyde, eugenol, carvacrol, methyl eugenol, cineol, linalool and caryophyllene. This plant also contains alkaloids, glycosides, flavonoids, saponins and citric, tartaric and malic acids (Gani, 1994). *Averrhoa carambola* Linn. fruit is very rich in vitamin-c, Oxalic acid and its Ca and P salts, acid potassium oxlate and vitamin A. It also contains protein and amino acids, young fruits contain tannins. *Leucas lavandulaefolia* R. & S. leaves contain an isopimarane rhamnoglucoside, linifolioside. *Coccinea cordifolia* Linn. leaves contain protein, fat, vitamin-c, sterols, β - sitosterol, phenolic compounds, triterpenoids, glycosidic constituents, alkaloids, cephalandrine A and B, alcohol, cephalendrol.

It is observed that leaf extract of Tulshi (*Ocimum sanctum* Linn.) has antifungal properties and it decreased the radial growth of *Neurospora crassa*. In most of the treatment sporulation of the fungus was reduced, radial growth decreased and colony became compressed as compared to the control. Antifungal properties of the plant extract are responsible for inhibition of growth of the fungus (Haque and Shamsi, 1997).

Presently scientist are interested to evaluate the antifungal activities of plant extracts against plant pathogenic fungi. Several workers have studied the antifungal properties of some medicinal plant extracts against some fungi. Ahmed and Sultana, 1984; Haque and Shamsi, 1996; Alice and Rao, 1987; Anwar *et al* 1994; Bashir and Rai, 1991; Bignami *et al* 1977; Carere *et al* 1978; Kishore *et al* 1982; Lokendra and Sharma, 1978; Miah *et al* 1990; Misra and Dixit, 1979; Nanir an Kadu, 1987; Sheikh and Agnihotri, 1972; Tiwari *et al* 1987.

Now a days a good number of fungicides we used in various purposes. Therefore a plane was made to induce mutation both morphological and biochemical by using leaf extract of Neem, Diathane-M45, UV radiation and ethyl methane sulphonate (keya, 1998).

The method of genetic analysis showed that there were genes on the same chromosomes or linked genes. Genes were assigned to specific linkage groups

on the basis of lineage to at least one marker, as evidenced by a significant excess of parental ditype over the non-parental ditype (Perkins, 1959).

In genetical research, it is accepted that the genes are localized in the chromosomes and it is obvious that each gene cannot segregate independently of all other because the number of genes and number of chromosomes do not compared (Davis and Serres, 1970).

The term linkage was first coined by Bateson and punnett in 1908. Morgan's concept about the linkage developed the theory of linear arrangement of genes in the chromosomes which become helpful for linkage of chromosomes. Seven linkage groups have been identified in *Neurospora crassa* in which haploid chromosome number is seven. The seven chromosome in *Neurospora crassa* must contain hundreds of genes. Each chromosome corresponds to a linkage groups.

Linkage was first demonstrated in *Neurospora crassa* by lindegren, 1933. Subsequently he published linkage map of linkage group-I (Lindegren, 1936) and II (Lindegren and lindegren, 1939). The seven Chromosomes of *Neurospora crassa* are identified by Arabic numerals (I-VII) in the chronological order in which they were discovered (Barratt *et al* 1954) Linkage study of the different mutant has one of the greatest significance for the living organisms, that it reduces the possibility of variability in gamets, unless crossing over occurs.

In common with many fungi, *Neurospora crassa* also possesses a growth phase in which genetically different haploid nuclear can exist with same cytoplasm in a hyphae. This phenomenon known as heterocaryosis is equivalent in many ways to the diploid but differs since more than one nucleus takes part in the determination of phenotype (Davis, 1966).

Nuclear ratios in heterocaryons and their effect on the phenotype have been investigated fairly extensively (Beadle and coonradt 1944; Baratt and Granjobst, 1949; Atwood and Mukai, 1953; Pittenger, 1964 and Davis 1966). Heterocaryons can originate by direct fusion between two hypae as they touch

and the subsequent flowing of nuclei from one hyphae to the other or by mutation of a nucleus in homocaryotic culture (Srb and Infanger, 1964).

Demonstration of heterocaryosis requires the microconidia or hyphal tip (Dodge 1942; Beadle and coonradt, 1944). While heterocaryon stability is controlled by a number of factors, including mating type (Whitehouse, 1949, Gross, 1952) and several incompatibility genes (Granjobst and Wilson, 1959; Wilson and Granjobst, 1966). Stable heterocaryons are readily obtainable in *Neurospora crassa* and provide a powerful tool for certain types of genetic analysis (Fincham, 1966; Granjobst, 1963). If two mutants complement each other, their mutation must be at different genes or at different sites.

A plan was made to study in detail few selected and interesting mutants for their morphological changes by studying the linear growth, mycelial weight, time required for germination, fertility, mating type and heterocaryosis in comparison with the wild type Ema.

Lastly the author also decided to study in detail the segregation and linkage of seven selected mutants.

MATERIALS

MATERIALS

The experimental materials was *Neurospora crassa* an Ascomycetes fungus. Culture Emerson 'a' (Em'a') (5297), Emerson "A" (EmA (5296) and markers were used for treating conidia with mutagens and Em'A' used for checking the mating types of the mutants and extracts. Markers were used for the study of linkage.

The following standard markers were used for the study of linkage.

The wild types and markers 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 Prof. Dr. T.I.M.A. Mozmader and Prof. Dr. Tahsina Rahim from Genetic Stock, Department of Botany, University of Dhaka.

1. (1) Wild types:
 - a) Emersons "A" (Em "A") (5296)
 - b) Emersons "a" (Em "a") (5297)

1(2). STANDARD MARKERS:

Table-1

Name of the Marker	No of the allele	Mating types	Position of the gene	Linkage group
1. Leu-3	R 156	A	IL	I
2. arg-5	27947	A	IIR	II
3. trp-1	10575	A	IIIR	III
4. trp-4	Y2198	A	IVR	IV
5. trp-5	A420	A	VR	V
6. trp-2	75001	A	VIR	VI
7. arg-10	B317	A	VII R	VII

1(3) MUTAGEN:

A. Physical Mutagen:

- a) UV irradiation of 254 nm.

B. Chemical Mutagen:

a) *Ocimum sanctum* Linn. (Tulshi), leaf extracts.

Scientific Name : *Ocimum sanctum* Linn.

Useful part : Leaf.

Chemical Constituents :

Tulshi leaves Yield a bright yellow essential oil containing phenols, aldehyde, eugenol, carvacrol, methyl eugenol, cineol, linalool, and caryophyllene. This plant also contains alkaloids, glycosides, flavonoids, saponins and citric, tartaric and malic acids. It has antifungal activities and is antiseptic (Gani, 1994).

b) **Fruit extracts of *Averrhoa carambola* Linn. (Kamranga).**

Scientific Name : *Averrhoa carambola* Linn..

Useful part : Fruit.

Chemical Constituents :

Fruit very rich in vitamin-c, Oxalic acid and its Ca and P. salts, acid potassium, oxalate and vitamin A. It also contains protein and amino acids. Young fruits contain tannins. Flowers contain rutin and quercetin glucosides. (Medicinal plants by Gani, 1994).

c) Leaf extract of *Leucas lavandulaefolia*. Rees. (Shetadron).

Scientific name : *Leucas lavandulaefolia* Rees.

Useful part : Leaf.

Chemical Constituents: An isopimarane rhamnoglucoside, linifolioside, has been isolated from this plant.

d) Leaf extracts of *Coccinea cordifolia* Linn. cogn. (Telakucha).

Scientific Name : *Coccinea cordifolia* (Linn.) cogn.

Useful Part : Leaf

Chemical Constituents:

Aerial Parts contain protein, fat, vitamin-c, sterols, β - Sitosterol, Phenolic compounds, triterpenoids, bitter glycosidic constituents, alkaloids, cephalandrine A and B. alcohol, cephalandrol, tritriacontane, heptacosane. Immature fruits contain a glycoside, cucurbitacin B, β - amyrin and lupeol. Fruits also contain β - sitosterol and taraxerol. Ripe fruits contain carotenoids. Roots contain lupeol, β - anyrin and β - sitosterol. (Medicinal plants by Gani,1994).

Table-2

Measurement of leaves extracts of some Medicinal plants.

Name of the medicinal plant	Useful part	Measurement of useful part in gm.
1. <i>Ocimum sanctum</i> Linn.	Leaf	100gm
2. <i>Averrhoa carambola</i> Linn.	Fruit	100gm
3. <i>Leucas lavandulaefolia</i> Rees.	Leaf	100gm
4. <i>Coccinea cordifolia</i> Linn.	Leaf	100gm

1(4). APPARATUS AND GLASSWARES WHICH WERE USED FOR THE PRESENT RESEARCH WORKS ARE AS FOLLOWS:

C. APPARATUS:

- (i) Autoclave
- (ii) Centrifuge
- (iii) Electric balance
- (iv) Fluorescence Microscope
- (v) Incubator
- (vi) Inoculation chamber.
- (vii) Refrigerator
- (viii) Ultra- violet lamp.

D. GLASS WARES:

	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 tube
6.	Test tubes	Pyrex	1.5"×36cm"	growth (race) tubes
7.	Petridishes	Pyrex	1.5" dia	Irradiation
8.	Petridishes	Pyrex	3" dia	Media
9.	Flasks (Erlenmeyer)	Pyrex	100ml	Media and water etc.
10.	Flasks (Erlenmeyer)	Pyrex	250ml	Media and water etc.
11.	Flasks (Erlenmyer)	Pyrex	500ml	Media and water etc.
12.	Flasks (Erlenmyer)	Pyrex	1000ml	Media and water etc.

E. CHEMICALS:**I. GENERAL:**

1. Agar (Bacto)
2. Ammonium nitrate (NH_4NO_3)
3. Ammonium Sulphate [NH_4SO_4]
4. Biotin [$\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_3\text{S}$]
5. Boric acid [H_3BO_3] an hydride
6. Calcium chloride ($\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$)
7. Chloroform [CHCl_3]
8. Citric acid [$\text{C}(\text{OH})(\text{COOH})(\text{CH}_2\text{COOH})_2 \cdot \text{H}_2\text{O}$]
9. Copper sulphate [$\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$]
10. Ferrous sulphate [$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$]
11. Glucose ($\text{C}_6\text{H}_{12}\text{O}_6$)
12. Magnesium sulphate [$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$]
13. Manganese sulphate [$\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$]
14. Hydrochloric acid [HCl]
15. Magnesium chloride [MgCl_2]
16. Methanol [CH_3OH]
17. Potassium nitrate [KNO_3]
18. Potassium di-hydrogen phosphate [KH_2PO_4]
19. Sodium chloride [NaCl]

20. Sodium Citrate [$\text{NaH}_2\text{C}_6\text{H}_5\text{O}_7, 2\text{H}_2\text{O}$]
21. Sodium Molybdate [$\text{Na}_2\text{MoO}_4, 2\text{H}_2\text{O}$]
22. Sorbose [$\text{OCH}_2(\text{CHOH})_3$]
23. Sucrose [$\text{C}_{12}\text{H}_{22}\text{O}_{11}$]
12. Zinc Sulphate [$\text{ZnSO}_4, 7\text{H}_2\text{O}$]

II. AMINO ACID & SUPPLEMENTS

1. Adenine
2. Arginine
3. Histidine
4. isoleucine and valine
5. Leucine
6. Lysine
7. Pyrimidine
8. tryptophan
9. Uridine

MEDIA

MEDIA

Wild type strains of *Neurospora crassa* can be grown on a suitable mixture of mineral salts in water plus a carbon source (Sucrose, glucose) and the vitamin biotin (Beedle and Tatum 1945, Anonymous 1966).

To prepare different media various stock solutions were needed. Conical flask was used for keeping the stock solution. The chemicals were added one after another and the addition of the next one was followed by the complete dissolution of the previous one. All the stock solution were kept in refrigerator. All media were sterilized in the autoclave at 121°C under 15lb pressure for 20 minutes.

A. COMPOSITION OF TRACE ELEMENTS SOLUTION PER 100ml

1.	Distilled water	95ml.
2.	Citric acid C(OH) (COOH) (CH ₂ COOH) H ₂ O	5g
3.	Zinc sulphate (ZnSO ₄ , 7H ₂ O)	5g.
4.	Ferrous sulphate (FeSO ₄ , 6H ₂ O)	1g.
5.	Copper sulphate (CuSO ₄ , 5H ₂ O)	0.25g
6.	Manganes sulphate (MnSO ₄ , H ₂ O)	0.65g.
7.	Boric acid anhydrous (H ₃ BO ₄)	0.05g.
8.	Sodium molybdate (NaMoO ₄ , 2H ₂ O)	0.05g.
9.	Chloroform (CHCl ₃)	1ml

B. COMPOSITION OF BIOTIN SOLUTION PER- 100ML.

- | | |
|--------------------|-------|
| 1. Distilled Water | 100ml |
| 2. Biotin | 10mg. |

C. VOGEL'S STOCK SOLUTION:

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

Composition of Vogel's stock solution per liter

1.	Distilled water	770ml
2.	Sodium citrate, 2H ₂ O	63.5g
3.	Potassium dihydrogen phosphate (KH ₂ PO ₄)	125g
4.	Ammonium Nitrate (NH ₄ NO ₃)	50g
5.	Magnesium Sulphate (MgSO ₄ , 7H ₂ O)	5g
6.	Calcium Chloride (CaCl ₂ , 6H ₂ O)	3.75g
7.	Trace elements solution	2.5ml
8.	Biotin solution	1.25ml
9.	Chloroform	2ml

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

D. WESTERGAARD'S STOCK SOLUTION:

It was essential for the preparation of Westergaard's crossing medium.

Composition of Westergaard's stock solution per liter.

1.	Distilled water	1000ml
2.	Potassium nitrate (KNO ₃)	4g
3.	Potassium dihydrogen Phosphate (KH ₂ PO ₄)	4g
4.	Magnesium sulphate (Mg SO ₄ 7H ₂ O)	4.1g
5.	Calcium chloride (CaCl ₂ , 6 H ₂ O)	0.8g
6.	Sodium Chloride (NaCl)	0.4g
7.	Biotin Solution	15 drops
8.	Chloroform (CHCl ₃)	2 ml .

E. STOCK SOLUTION OF AMINO ACIDS :

This solution was required for the preparation of supplemented medium. 100 mg of amino acid was dissolved in 100 ml of sterilized distilled water. The stock solution was named after the amino acid used. eg. tryptophen solution, adenine solution, leucine solution etc. In case of culture and crossing media the amino acid supplements were added at the time of their preparation. The amino acid solution was also kept in 100ml conical flask. For frequent use solution were kept in test tubes fitted with sterilized pipettes. The solutions in the pipettes were used drop wise.

F. 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 10ml of medium was taken in large test tubes (6" and 2-4 ml of medium was taken in 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, arginine medium etc.

G. COMPOSITION OF VOGEL'S MINIMAL MEDIUM (V.M) PER LITER FOR CULTURING.

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

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

H. VOGEL'S MINIMAL LIQUID MEDIUM:

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

It was used for determining mycelial growth of the organism. The medium was supplemented where necessary.

I. MEDIUM OF DETERMINING LINEAR GROWTH:

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

This media was supplemented where necessary.

J. MEDIA FOR PLATING :

Sorbose minimal medium was used for plating. It restricts growth so that colonies do not run into each other which could happen in VM. The rapid linear growth rate of *Neurospora crassa* was a serious drawback when individual colonies and single spore needed to be isolated. The problem was circumvented by the finding that when creating chemicals, notably the ketohexose sorbose had been added to the culture media 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, Pittenger, 1964). For that reasons supplemented sorbose minimal medium as well as non supplemented sorbose minimal medium were used for plating. This medium was also used for minimal best of wild type and mutant.

K. COMPOSITION OF SORBOSE MINIMAL (SM) MEDIUM PER LITRE :

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

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

L. MEDIUM FOR CROSSING:

Westergaard's crossing medium.

This media was used for crossing. It restricts the vegetable growth but enhances sexual reproduction.

Composition of westergaard's crossing medium per liter:

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

Necessary supplements were used while mutants with wild or markers were crossed and mutants with mutant were crossed.

The filter papers measuring 1.5" × 2.5" were folded three times to make two furrows and introduced into the test tube (8"). Then 6-8 ml of the medium was poured in each crossing tubes. The medium remained in the furrow of the crossing tubes. The medium remained in the furrow of the folded paper when the tubes were slanted.

For case of fertile cross, sucrose was used 0.2g per 100ml and the percentage of the supplement was increased to 50 mg per 100 ml. (Suyama *et al* 1958) It had the following composition for 100 ml.

M. COMPOSITION OF SUYAMA'S CROSSING MEDIUM.

- | | | |
|----|------------------------------|--------|
| 1. | Distilled water | 960 ml |
| 2. | Westergaard's stock solution | 250 ml |
| 3. | Agar | 20g |
| 4. | Sucrose | 2g. |

This media was supplemented where necessary.

METHODS

METHODS

1. STOCK MAINTAINING:

The Stock of the cultures of the mutants, markers, wild types (Em'a' and Em'A') were maintained in stock tubes containing 5 ml medium with necessary supplements (in case of mutants and markers). The wild type could grow in Vogel's minimal medium without any supplement. After incubation period, when cultures produce sufficient conidia were preserved in a refrigerator at $\pm 40^{\circ}\text{C}$. The cultures were found to produce sufficient conidia within 4 to 5 days.

2. SUB CULTURING:

Sub culturing was important to obtain fresh cultures and for preservation. Generally $2\frac{1}{2}$ " - 3" tubes were used for this purpose. 2 ml sterilized Vogel's minimal medium or Vogel's supplemented medium (where supplement was necessary) were used for sub culturing. Wild type (Ema and Ema) and morphological mutant were subcultured in Vogel's minimal medium. Biochemical mutants and markers were subcultured in supplemented VM. Particular amino acid supplements were used for particular biochemical mutants and markers.

Subculturing was done in a sterilized inoculation chamber with at most care to avoid any kind of infection. The chamber, inoculation needle was sterilized with rectified spirit.

The inoculation needle was sterilized by heating over the flame of a 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.

3. SPONTANEOUS MUTATION IN NEUROSPORA CRASSA:

Spontaneous mutation in *Neurospora crassa* is very infrequent in nature (Stadler *et al* 1993). It was observed if there was any spontaneous mutation in *neurospora crassa*. There were several steps for finding out the spontaneous mutation in *neurospora crassa*.

a) OBTAINING OF FRESH CULTURE OF Ema:

For obtaining of fresh culture Ema was subcultured four times offer four days interval.

b) CENTRIFUGATION:

10 ml of sterilized distilled water was taken in a centrifuge tube. One loop of conidia from fresh culture of 4-6 days old was taken in the solution with a sterilized loop needle and shaken well to make homogenous suspension of conidia. Then the suspension was centrifuged for 10 minutes.

c) PREPARATION OF SUSPENSION:

After centrifugation the distilled water was poured out from the centrifuge tube. So that the conidia could remain at the bottom of the test tube. Then 10ml sterilized distilled water was added and was shaken well.

d) PLATING AND INCUBATION:

1 drop of conidial suspension was taken in sterilized petridishes (60) with sterilized pipette and 10 ml of molten SM medium at 47°C was added in each petridish. The conidial suspension and SM media were mixed by shaking gently. Then plates were incubated at 25°C for growth of conidia.

e) ISOLATION:

After growth of conidial colonies in SM petriplates, Single conidial colonies were isolated in fresh and sterilized VM in test tubes with isolating needle. The isolates were incubated at 25°C for proper growth.

f) OBSERVATION:

After 5 days the cultures were observed and compared with Ema if there was any morphological changes such as mycelia, conidia, colour etc. There was no change in morphology.

4. STUDY OF MUTAGENIC EFFECT OF EXTRACTS OF *Ocimum Sanctum* Linn. (TULSHI), ON *Neurospora crassa*.

- a) Preparation of solution of different concentrations of leaf extract of *Ocimum sanctum* Linn. (Tulshi).

At first 100 gm. of fresh leaves of *Ocimum sanctum* 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. After air drying the leaves were grinded in an electric grinder.

A paste was made with the help of a grinder. The paste was filtered 2-3 times with the help of sterilized thick cotton cloth and finally it was filtered with the help of centrifuge machine.

The pure leaves extract was marked by T_p . 100% crude extracts and 10ml of sterilized distilled water were mixed well with 10ml crude extracts and the 50% concentration was marked as T_1 . and 20ml of sterilized distilled water were mixed well with 10ml crude extracts and the 33.33% concentration was marked as T_2 . and 30 ml of sterilized distilled water were mixed well with 10ml crude extracts and the 25% concentration was marked as T_3 . and 40ml of sterilized distilled water were mixed well with 10ml crude extracts and the 20% concentration was marked as T_4 . 10ml of T_p , T_1 , T_2 , T_3 , T_4 , were taken in separate tubes.

Each concentration was taken in duplicate and one set was sterilized in the autoclave at 121°C under 1516 pressure for 20 minutes and other set was kept at normal temperature of the laboratory.

50gm leaves of Tulshi were washed with sterilized distilled water.

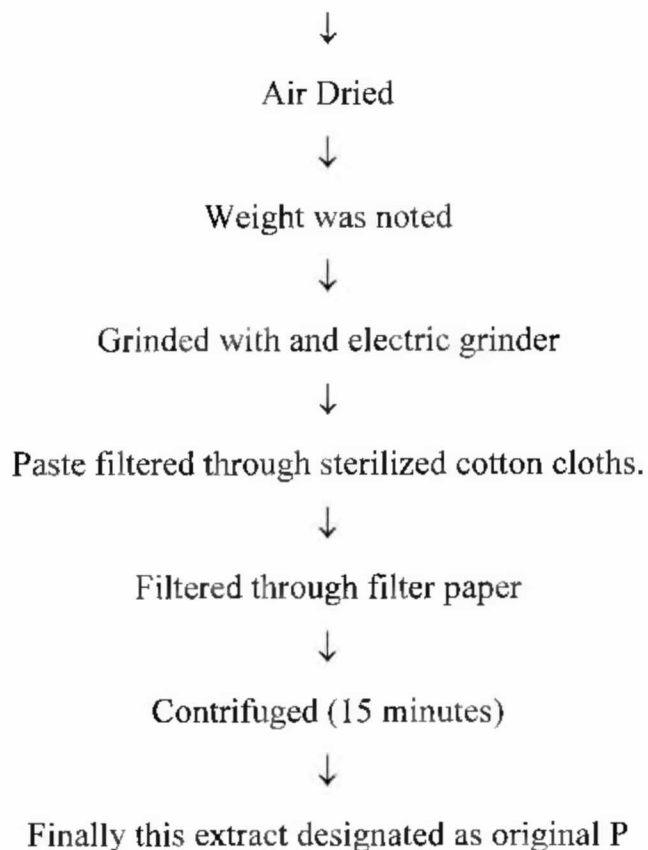


Fig :-1. Preparation of solution of different concentrations of leaf extract.

b) EFFECT OF LEAF EXTRACT OF *Ocimum sanctum* Linn. (TULSHI), ON THE GROWTH OF *Neurospora Crassa*:

At first the petridishes were marked as O_p (100%), O₁ (50%), O₂ (33.33%), O₃ (25%), O₄ (20%) and O₀ (no extract only VM) with the help of glass marking pen. 1 ml of leaves extracts solution of different concentrations were taken in separate sterilized petridishes with the help of sterilized pipette. 10ml of molten VM plating medium was added in each petridish and the petridishes were shaken gently for through mixing of the extracts. As for control VM plating medium was taken in each sterilized petridish. When the petridish was cooled 4 days old Em'a' was inoculated at the centre of the petridishes inside the inoculation chamber to prevent contamination. Test for both control and the leaves extract concentration was done in duplicate.

The petridishes were kept in the incubator at 25°C and observed after 18, 20, 24 and 42 hours.

c) **TREATING OF Em'a' WITH EXTRACT OF LEAF OF *Ocimum sanctum* linn:**

i) **OBTAINING OF FRESH CULTURE OF Em'a':**

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

ii) **STERILIZATION:**

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

iii) **CENTRIFUGATION:**

5 ml of pure (100%) leaf extract of *Ocimum sanctum* linn. were taken into 4 centrifuge tubes marked T₁, T₂, T₃, and T₄ with the help of glass marking pen. 1 loop of conidia of Em'a' was given to T₁, T₂, T₃, T₄ tubes and waited for 1 hour, 2 hours, 4 hours and 6 hours previously. The tubes and waited for 1 hour, 2 hours, 4 hours and 6 hours previously. The tubes were shaken gently for homogeneous solution. The tubes with suspension were then placed into centrifuge machine and centrifuged for 10 minutes.

(iv) **FILTRATION:**

The solutions of the centrifuge tubes were poured out carefully.

So that conidia could remain in the bottoms of the tubes. 5 ml of sterilized distilled water was taken into each tubes 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.

(v) PREPARATION OF SUSPENSION WITH TREATED CONIDIA:

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

(vi) PLATING OF TREATED CONIDIA:

1 drop of the suspension was taken in each sterilized Petri dish was marked as T_p , T_1 , T_2 , T_3 , T_4 , T_0 previously and the suspension was taken accordingly. 10 ml of molten SM at $45-47^{\circ}\text{C}$ 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.

(vii) INCUBATION:

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

(viii) ISOLATION:

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

(ix) INCUBATION:

The cultures were incubated at a temperature of 25°C .

(x) CLASSIFICATION:

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

5. **STUDIES ON MUTAGENIC EFFECT OF FRUIT EXTRACT OF *Averrhoa Carambola* Linn. (KAMRANGA) ON *Neurospora crassa*.**

a) **PREPARATION OF SOLUTION OF DIFFERENT CONCENTRATIONS OF FRUIT EXTRACT OF *Averrhoa carambola* Linn. :**

At first 100 gm of fresh fruit of *Averrhoa carambola* Linn. was measured with the help of a balance. The fruit was washed with tap water 2-3 times and finally it was washed with distilled water. A paste was made with the help of a grinder. The paste was filtered 2-3 times with the help of sterilized thick cotton cloth and finally it was filtered with the help of centrifuge machine.

The pure fruit extract was marked by Kp. 100% crude extracts and 10ml of sterilized water were mixed well with 10ml crude extracts and the 50% concentration was marked as K₁ and 20ml of sterilized distilled water were mixed well with 10ml crude extracts and the 33.33% concentration was marked as K₂. and 30ml of sterilized distilled water were mixed will with 10ml crude extracts and the 25% concentration was marked as K₃. and 40ml of sterilized distilled water were mixed well with 10ml crude extracts and the 20% concentration was marked as K₄. 10ml of Kp, K₁, K₂, K₃, K₄ solution were taken in separate tubes. Each concentration was taken in duplicate and one set was sterilized in the autoclave at 121°C under 1516. pressure for 20 minutes and other set was kept at normal temperature of the laboratory.

b). **EFFECT OF FRUIT EXTRACT OF *Averrhoa Carambola* Linn. (KAMRANGA), ON THE GROWTH OF *Neurospora crassa* . :**

At first the Petridishes were marked as O_k (100% extract, O₁ (50%ext.), O₃ (25% ext.) and O₀ (No extract only VM) with the help of glass marking pen. 1ml of leaves extracts solution of different concentrations were taken in sparate sterilized petridishes with the help of sterilized pipette. 10ml of molten VM

plating medium was added in each petridish and the petridishes were shaken gently for through mixing of the extracts. As for control VM plating medium was taken in each sterilized petridish. When the petridish was cooled 4 days old Em'a' was inoculated at the centre of the petridishes inside the inoculation chamber to prevent contamination. Test for both control and the fruit extract concentration was done in duplicate.

The petridishes were kept in the incubator at 25^{0c} and observed after 20, 22 with extract of fruit of *Averrhoa carambola* Linn. and 40 hours.

(c) TREATING OF Em'a' WITH EXTRACT OF FRUIT OF *Averrhoa carambola* Linn. :

(i) OBTAINING OF FRESH CULTURE OF Em'a':

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

(ii) STERILIZATION :

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

(iii) CENTRIFUGATION:

5ml of fruit extract of *Averrhoa carambola* Linn. were taken into 5 centrifuge tubes marked K₁, K₂, K₃, and K₄, with the help of glass marking pen. 1 loop of conidia of Em'a' was given to K₁, K₂, K₃, K₄, tubes and waited for 1 hour, 2 hours, 4 hours and 6 hours previously. The tubes were shaken gently for homogenous solution.

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

(iv) FILTRATION:

The solutions of the centrifuge tubes were poured out carefully. So that conidia could remain in the bottoms of the tubes. 5ml of sterilized distilled water was taken into each tubes 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.

v) PREPARATION OF SUSPENSION WITH TREATED CONIDIA:

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

vi) PLATING OF TREATED CONIDIA:

1 drop of the suspension was taken in each sterilized petridish. The petridish was marked as k_p , k_1 , k_2 , k_3 , k_4 , k_0 previously and the suspension was taken accordingly. 10ml of molten SM at $45-47^{\circ}\text{C}$ was added to each petridish. The temperature of the media did not exceed 47°C . The suspension and media were mixed well by shaking the petridish gently. The whole experiment was done in the chamber to avoid contamination.

vii) INCUBATION:

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

viii) ISOLATION:

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

ix) INCUBATION:

The cultures were incubated at a temperature of 25°C .

x) CLASSIFICATION:

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

xi) GROWTH TEST OF DIFFERENT MORPHOLOGICAL MUTANTS ON SM, VM (SUCROSE) AND VM (GLUCOSE):

Growth tests were made in sterilized petridishes containing sorbose minimal medium, Vogel's minimal medium (Sucrose) and Vogel's minimal medium (glucose was used in lieu of sucrose). The plates were divided into 24 compartments by glass marking pencil. Then conidia from a particular mutant were put in one compartment, 23 compartment contained conidia from different mutants and one contained wild type (Ema) as control.

6. STUDIES ON MUTAGENIC EFFECT OF LEAF EXTRACT OF *Leucas lavandulaefolia* Rees.(SHETADRON) ON *Neurospora crassa*.

a) PREPARATION OF SOLUTION OF DIFFERENT CONCENTRATIONS OF LEAF EXTRACT OF *Leucas lavandulaefolia* Rees:

At first 100 mg of fresh leaves of *Leucas lavandulaefolia rees.* 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. A paste was made with the help of a grinder. The paste was filtered 2-3 times with the help of sterilized thick cotton cloth and finally it was filtered with the help of centrifuge machine.

The pure leaf extract was marked by Sp. 100% crude extracts and 10 ml of sterilized distilled water were mixed well with 10 ml crude extracts and the 50% concentration was marked as S₁ and 30 ml of sterilized distilled water were mixed well with 10 ml crude extracts and the 25% concentration was marked as S₃. 10 ml of S_p, S₁, S₃ solution were taken in separate tubes. Each concentration was taken in duplicate and one set was kept at normal temperature and other set was kept in refrigerator.

b) EFFECT OF LEAF EXTRACT OF *Leucas leavandulaefolia* Rees. (SHETADRON), ON THE GROWTH OF *Neurospora crassa*:

At first the petridishes were marked as O_p (100% extract), O₁ (50% extract), O₃ (25% extract), O₀ (No extract) with the help of glass marking pen. 1ml of leaves extracts solution of different concentrations were taken in separate sterilized petridishes with the help of sterilized pipette. 10 ml of molten VM plating medium was added in each petridish and the petridishes were shaken gently for through mixing of the extracts. As for control VM plating medium was taken in each sterilized petridish. When the petridish was cooled 4 days old Em'a' was inoculated at the centre of the petridishes inside the inoculation chamber to prevent contamination. Test for both control and the leaf extract concentration was done in duplicate.

The petridishes were kept in the incubator at 25⁰C. and observed after 18,20 and 24 hours.

c) TREATING OF Em'a' With Extract of Leaf of (*Leucas leavandulaefolia* Rees):

i) OBTAINING OF FRESH CULTURE OF Em'a':

For obtaining of fresh culture Em'a' was subcultured 4 times after an interval of 4 days.

In each case culture of 5 days old were used for treating conidia.

ii) STERILIZATION:

Inoculation chamber, needle, hands, centrifuge tubes and centrifuge machine were sterilized with rectified spirit. The centrifuge tubes were then washed with sterilized distilled water. Media, Petridishes, tubes with pipettes and distilled water were sterilized previously in the autoclave at 121⁰C, 15 lb pressure for 20 minutes.

iii) CENTRIFUGATION:

5 ml of leaf extract of *Leucas leavandulaefolia rees.* were taken into 5 centrifuge tubes marked S₁, S₂, S₃, S₄ with the help of glass marking pen. 1 loop of conidia of Em'a' was given to S₁, S₂, S₃, S₄ tubes and waited for 1 hour, 2 hours, 4 hours and 6 hours previously. The tubes were shaken gently for homogeneous solution. The tubes with suspension were then placed into centrifuge machine and centrifuged for 10 minutes.

iv) FILTRATION:

The solutions of the centrifuge tubes were poured out carefully. So that conidia could remain in the bottoms of the tubes. 5 ml of sterilized distilled water was taken into each tubes 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.

v) PREPARATION OF SUSPENSION WITH TREATED CONIDIA:

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

vi) PLATING OF TREATED CONIDIA:

1 drop of the suspensions was taken in each sterilized petridish. The petridish was marked as S_p, S₁, S₂, S₃, S₄, S₀ previously and the suspension was taken accordingly. 10 ml of molten SM at 45-47°C was added to each petridish. The temperature of the media did not exceed 47°C. The suspension and media were mixed well by shaking the petridish gently. The whole experiment was done in the chamber to avoid contamination.

vii) INCUBATION:

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

viii) ISOLATION:

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

ix) INCUBATION:

The cultures were incubated at a temperature of 25°C.

x) CLASSIFICATION:

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

xi) GROWTH TEST OF DIFFERENT MORPHOLOGICAL MUTANTS ON SM, VM (SUCROSE) AND VM (GLUCOSE):

Growth tests were made in sterilized petridishes, containing sorbose minimal medium, Vogel's minimal medium (Sucrose) and Vogel's minimal medium (glucose was used in lieu of sucrose). The plate were divided in to 24 compartments by glass marking pencil. Then conidia from a particular mutant were put in one compartment, 23 compartment contained conidia from different mutants and one contained wild type (Ema) was control.

7. STUDIES ON MUTAGENIC EFFECT OF LEAF EXTRACT OF *Coccinea cordifolia* Linn. (TELAKUCHA), ON *Neurospora crassa*.

a) PREPARATION OF SOLUTION OF DIFFERENT CONCENTRATIONS OF LEAF EXTRACT OF *Coccinea cordifolia* Linn:

At first 100 gm of fresh leaves of *coccinea cordifolia* 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. A paste was made with the help of a grinder. The paste was filtered 2-3 times with the help of sterilized thick cotton cloth and finally it was filtered with the help of centrifuge machine.

The pure leaf extract was marked by C_p . 100% crude extracts and 10ml of sterilized distilled water were mixed well with 10ml crude extracts and the 50% concentration was marked as C_1 and 30ml of sterilized distilled water were mixed well with 10ml crude extracts and the 25% concentration was marked as C_3 . 10ml of C_p , C_1 , C_3 solution were taken in separate tubes. Each concentration was taken in duplicate and one set was kept at normal temperature and other set was kept in refrigerator.

b) EFFECT OF LEAF EXTRACT OF *Coccinea cordifolia* Linn. (TELAKUCHA), ON THE GROWTH OF *Neurospora crassa*:

At first the petridishes were marked as O_p (100% crude extract), O_1 (50% extract), O_3 (25% extract), O_0 (no extract) with the help of glass marking pen. 1ml of leaves extracts solution of different concentrations were taken in separate sterilized petridishes with the help of sterilized pipette. 10 ml of molten VM plating medium was added in each petridish and the petridishes were shaken gently for through mixing of the extracts. As for control VM plating medium was taken in each sterilized petridish. When the petridish was cooled 4 days old Em'a' was inoculated at the centre of the petridishes inside the inoculation chamber to prevent contamination. Test fro both control and the leaf extract concentration was done in duplicate.

The petridishes were kept in the incubator at 25°C and observed after 18,20 and 24 hours.

c) **TREATING OF Em'a' WITH EXTRACT OF LEAF OF
Coccinea cordifolia Linn:**

i) **OBTAINING OF FRESH CULTURE OF Em'a':**

For obtaining of fresh culture Em'a' was subcultured 4 times after an interval of 4 days.

In each case culture of 5 days old were used for treating conidia.

ii) **STERILIZATION:**

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

iii) **CENTRIFUGATION:**

5 ml of leaf extract of *coccinea cordifolia* linn. were taken into 5 centrifuge tubes marked C₁, C₂, C₃, C₄ with the help of glass marking pen. 1 loop of conidia of Em'a' was given to C₁, C₂, C₃, C₄ tubes and waited for 1 hour, 2 hours, 4 hours, and 6 hours previously. The tubes were shaken gently for homogeneous solution. The tubes with suspension were then placed into centrifuge machine and centrifuged for 10 minutes.

iv) The solutions of the centrifuge tubes were poured out carefully. So that conidia could remain in the bottoms of the tubes. 5 ml of sterilized distilled water was taken into each tubes and centrifuged for 3 minutes. The upper liquid was poured out. The some procedure of pouring out upper liquid, adding water and centrifugation was repeated 2 times.

v) PREPARATION OF SUSPENSION WITH TREATED CONIDIA:

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

vi) PLATING OF TREATED CONIDIA:

1 drop of the suspension was taken in each sterilized petridish. The petridish was marked as C_p, C₁, C₂, C₃, C₄, C₀ previously and the suspension was taken accordingly. 10 ml of matter SM at 45-47°C was added to each petridish. The temperature of the media did not exceed 47°C. The suspension and media were mixed well by shaking the petridish gently. The whole experiment was done in the chamber to avoid contamination.

vii) INCUBATION:

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

viii) ISOLATION:

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

ix) INCUBATION:

The cultures were incubated at a temperature of 25°C.

x) CLASSIFICATION:

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

xi) GROWTH TEST OF DIFFERENT MORPHOLOGICAL MUTANTS ON SM, VM (SUCROSE) AND VM (GLUCOSE):

Growth tests were made in sterilized petridishes containing sorbose minimal medium, Vogel's minimal medium (sucrose) and Vogel's minimal medium (glucose was used in lieu of sucrose). The plates were divided in to 24 compartments by glass marking pencil. Then conidia from a particular mutant were put in one compartment, 23 compartment contained conidia from different mutants and one contained wild type (Ema) as control.

8. ARTIFICIAL INDUCTION OF MUTATION BY UVIRRADIATION.

a) OBTAINING FRESH CULTURE:

Fresh cultures were made by subculturing the Ema 3-4 times.

b) PREPARATION OF CONIDIAL SUSPENSION AND IRRADIATION:

10 ml of suspension was made with 5 days old conidia of Ema and sterilized distilled water. 2ml of suspension was transferred to 3 small sterilized petridishes and irradiated with UV light having wave length of 254nm for 2, $2\frac{1}{2}$ & 3 minutes. Radiation was given at a distance of 15cm.

The suspension was continuously rotated during irradiation with an wooden handle to ensure an equal dose for all conidia. After completion of irradiation the suspension was stored at room temperature in the dark for 2 hours to prevent photo reactivation.

c) PLATING OF THE IRRADIATED CONIDIA:

After 2 hours the irradiated suspension was taken in petridishes. 20 petridishes were taken for each suspension and 1-2 drops of suspension were given in petridishes. 10ml molten SM supplemented with leucine, lysine, adenine, arginine, histidine and tryptophan was used for plating.

d) INCUBATION:

The plates were incubated at 25⁰C. observation were taken at interval of 24 hours until some colonies were visible.

e) ISOLATION:

Separated colonies were isolated into VM supplemented tubes with lousine, lysine, arginine, adonine, histidine and tryptophan medium and incubated at 25⁰C.

f) OBSERVATION:

Among isolates some showed morphological abnormalities. After 5-7 days those were classified by comparing their characters with will type Ema.

g) TEST FOR BIOCHEMICAL MUTANTS:

All mutants were tested on SM and SM supplemented plates for biochemical mutants.

9. SELECTION OF SOME MORPHOLOGICAL MUTANTS:

Mutants from different groups were subcultured three 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 growth, conidial germination, crossing, linkage and protein test etc.

10. STUDY OF LINEAR GROWTH:

Linear growth of selected mutants were measured in race tube, made of long pyrex glass tube. The horizontal portion was kept 36cm long and inner diameter of about 15mm. The two sides which were bent up one at an angle of 45° and opposite side at 90° angle with a lower projection of 1cm. Those tubes were held up right on a flat surface with a holders. Flat portion of the tube were half filled with the solid Vogel's minimal medium containing 4% agar. Mutants and Ema were inoculated at one end of the medium of the tube. Linear growth of the tube were measured in cm at 6 hours interval.

11. STUDY OF MYCELIAL GROWTH AND WEIGHT:

It was done in liquid Vogel's minimal medium. Selected mutants and Ema was inoculated in the medium of the flask. Each flask contained 50ml medium. To prevent the formation of conidia flask were shaken continuously. Weight of the empty filter papers was taken. When the culture was 72 hours old, flasks with culture were boiled in water for 2 minutes and were filtered with filter paper of known weight. The filter papers with mycelia were dried in an oven and the weight of filter papers with culture were taken. Those were done with control.

12. TEST FOR HETEROKARYON COMPATIBILITY:

Heterokaryon test were done in order to classify the mutants basing on their positive (complementation) or negative (Non- complementation). The test were done among the mutants of same mating type. And for these tests always fresh cultures of 4 to 6 days and fresh medium were used a suggested by reverend guide for better accuracy.

Tests for Heterokaryon compatibility were made on Vogel's minimal both liquid and solid medium in small sized test tubes. Before doing the tests the mutants were subcultured in order to keep the stock and then the test were done. Generally two sets of test were done on control set and one Heterokaryon set. Separate control set for each mutants were maintained to see whether the mutants used grow in VM or not. Both control and Heterokaryon test done in Duplicate.

In Heterokaryon tests in solid medium few conidia from a mutant were placed in the tubes in two places and then conidia from another mutant were placed exactly at the same places as the conidia from the first mutant were placed.

CHECKING THE MATING TYPE OF THE EXTRACTS:

The selected extracts were chosen and their mating type was determined by crossing them with both will type Ema and EmA. If perithecia formed with Ema the mating type of the extracts would be 'A' and if perithecia formed with EmA it would be Ema.

13. CROSSING:

Neurospora crassa strains are heterothallic with two mating types 'A' and 'a'. Sexual fruiting bodies called perithecia are produced only when two mating types are brought together on a suitable media (Sher and Dodge 1927; Westergaard and Mitchell 1947, Anonymous 1966). Westergaard's crossing medium was usually used for crossing. In some cases, when the crosses were sterile i.e. Perithecia did not form, Then Suyama's crossing medium was used (Suyama *et al* 1958).

Crossing was made in a test tube (6") containing 5-7ml of Westergaard's crossing medium. One strain was put on one troughs of the filter paper pleat and the other was put on other trough of the pleat. The crosses were then incubated at 25°C which is the optimum temperature for sexual reproduction.

Fertile mutants formed perithecia within 5-8 days and in rare cases after 10-12 days of incubation. It was observed that the more delay in the formation of perithecia, the less is the number of perithecia formed in a cross.

Further the greater the concentration of amino acid supplement the earlier was the formation of perithecia and also the higher number of perithecia. The perithecia generally liberated spores with in 15-30 days.

The mutants were cross with wild types Em'a' and Em'A' for checking the mating types and they were crossed with biochemical markers of seven linkage groups for the determination of linkage group.

i) METHOD FOR THE DETERMINATION OF LINKAGE GROUP:

The method of determination of linkage group has been described under the following title:

a) SUBCULTURING OF INDUCED MUTANTS AND MARKERS:

The mutants and markers were subcultured four times after 4 days interval to improve their fertility.

b) CROSSING:

Mutants were crossed with seven markers and incubated at 25⁰C.

c) OBSERVATION OF THE CROSSES:

The fertile cultures formed perithecia in 5-8 days and shedded spores with in 15-20 days.

d) SPREADING OF SPORES ON SM PLATES:

About 10 ml sterilized medium preserved in 20 ml tube was taken in the clean, dried and sterilized petridish. The medium was supplemented with the amino acid according to the requirement. A spreading needle made of glass rod bent twice at right angles was boiled in water and kept for spreading. One drop of sterilized water was taken on the medium of the petridish. A loop needle were burnt red hot and then cooled in sterilized distilled water. A loop of spores were taken from the desired cross with the help of this needle and were transferred to water drop on the SM plate. The spreading needle was then cooled in sterilized distilled water plate and the spores were uniformly spread with it. The needle was again boiled for sterilization. The name of the cross was written on the upper lid of the petridish. The prepared petridish was given a heat shock at 58⁰C for 50 minutes and was then incubated at 25⁰C for 12-16 hours.

e) COUNTING OF SPORES FROM SM PLATE:

After incubation period the spreading SM plate were examined under stereoscopic microscope and the germinating spores, growing spores, non growing spores were counted. Parallel lines were drawn with a glass marking pencil on the back of the lower lid of the petridish. The spores were counted from one end of the space with in the two parallel lines to the other and by slowly moving the plate in one direction. The next space was then focussed on completion of the previous one and the spores were counted by moving the plate in the direction opposite of the preceding one.

f) ISOLATION OF SINGLE SPORES:

On completion of counting the spores (both growing and germinating) separately the plate was opened by taking the upper lid and the growing spores were isolated with the help of an isolating needle in the VM supplemented tubes. The tubes were incubated at 25⁰C for growth.

g) CHECKING THE ISOLATES:

After 4 days isolates were analyzed. The ratio between the mutant isolates and wild like isolates was calculated.

h) PLATE TEST OF THE EXTRACTS OF MUTANT:

For linkage study the isolates were tested on SM and supplemented SM plates.

EXPERIMENT AND RESULTS

EXPERIMENT AND RESULTS

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

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 colonies were isolated.

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

2. STUDY OF MUTAGENIC EFFECT OF EXTRACTS OF KRISHNA TULSHI (*Ocimum sanctum* Linn.) ON *Neurospora crassa*.



Fig-2: Photograph of Tulshi (*Ocimum sanctum* Linn)

Different concentrations of Tulshi solutions were used to test the mutagenic effect on *Neurospora crassa*. Fixed concentrations had appropriate mutagenic effect on *N. crassa*.

- (a) Preparation of different extracts of *Ocimum sanctum* Linn. (Krishna Tulshi), Leaves.

Different concentration of leaf extract of Tulshi solutions with sterilized distilled water were prepared.

Table-3

Different concentrations of *Ocimum sanctum* Linn. (Tulshi) extract solution.

Name of the solutions	Amount of crude extracts	Amount of sterilized distilled water	Concentration of solution in %
T _p	10 ml pure leaves extracts	0	100% crude extract
T ₁	10 ml crude extracts	10 ml sterilized distilled water	50% crude extract
T ₂	10 ml crude extracts	20 ml sterilized distilled water	33.33% crude extract
T ₃	10 ml crude extracts	30 ml sterilized distilled water	25% crude extract
T ₄	10 ml crude extracts	40 ml sterilized distilled water	20% crude extract

The prepared solutions were kept separately in sterilized test tubes.

- (b) **EFFECT OF EXTRACTS OF *Ocimum sanctum* Linn. (TULSHI) ON THE GROWTH OF *N. crassa*.**

For testing the effect, 1 ml of different solutions were taken separately on the sterilized petridishes with the help of sterilized pipette. The petridishes were marked as Op (100% crude extract), O₁, (50% extract), O₃ (25% extract), O₄ (20% extract) and O₀ (no extract). 10ml of molten VM was added in each petridish. When media became solid Ema was inoculated at the centre of the media. Plates were incubated at 25°C and measured the radial growth after 18, 20, 24, 40 and 44 hours. Following were the results.

Table-4

Radial growth of Ema on VM containing *Ocimum sanctum* Linn. (Tulshi).
extracts.

Plate No. and concentration of the solution	Observation after the following hours growth in mm.									
	18 hours		20 hours		24 hours		40 hours		44 hours	
	Growth in mm.	Mean in mm.	Growth in mm.	Mean in mm.	Growth in mm.	Mean in mm.	Growth in mm.	Mean in mm.	Growth in mm.	Mean in mm.
Op (100% crude extract)	6	6.5	7	7.5	9	9.5	13	13.5	15	15.5
	7		8		10		14		16	
O ₁ (50% crude extract)	7	7.5	9	9.5	11	11.5	14	14.5	16	16.5
	8		10		12		15		17	
O ₃ (25% crude extract)	10	10.5	11	11.5	13	13.5	16	16.5	17	17.5
	11		12		14		17		18	
O ₄ (20% crude extract)	15	15.5	18	18.5	23	23.5	40	41	45	46
	16		19		24		42		47	
O ₀ (No extract, only VM)	17	17.5	19	19.5	25	25.5	43	44	53	(54) over
	18		20		26		46		55	

Extracts of Krishna *Ocimum sanctum* Linn. (Tulshi) Leaves showed antifungal properties, So it restricted the growth of *Neurospora crassa*.

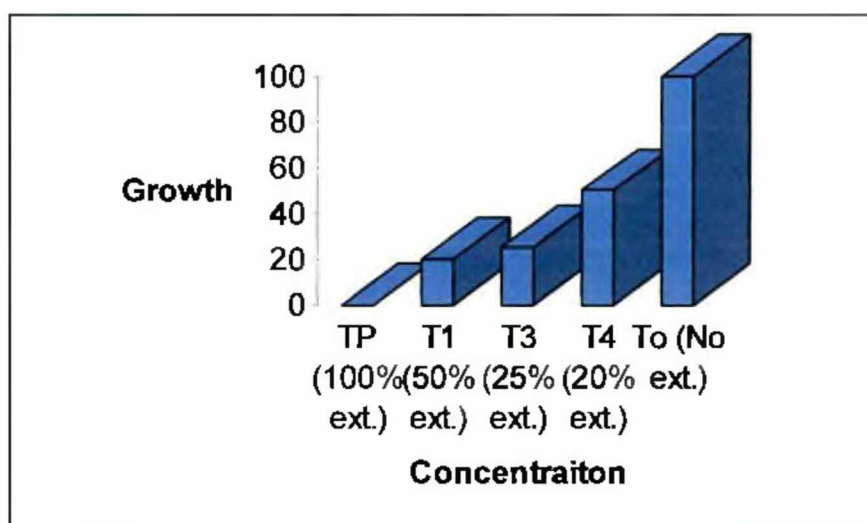


Fig:- 3. Bar graph of Radial growth of Ema with leaf extracts of Tulshi.



Fig-4: Comparative radial growth of Ema on VM containing leaf extract of Tulsh (*Ocimum sanctum* Linn.).

c). Treating of Ema with extracts of Tulsh (*Ocimum sanctum* Linn.) Leaves

Pure (100%) leaves extracts of Tulsh (*Ocimum sanctum* Linn.) were chosen for treating Ema. The treating time were 1 hours, 2 hours, 4 hours and 6 hours previously.

Classification of Talshi induced mutant :

Group-A: *ropy*:

Growth is checked. The hyphae are colourless and look like *rope*, conidia orange and less in number 12 mutants of this group are as follows:

roT₄-93, *roT₃*-117, *roT₃*-173, *roT₂*-187, *roT₄*-212, *roT₄*-232, *roT₄*-283, *roT₃*-297, *roT₄*-314, *roT₄*-327, *roT₁*-387, *roT₄*-400,

Group-B: *albino*

Mycelia and conidia are colourless. The following 10 mutants fall into this group. *alT₃*-17, *alT₄*-34, *alT₂*-69, *alT₁*-77, *alT₄*-98, *alT₃*-124, *alT₄*-153, *alT₄*-209, *alT₄*-272, *alT₃*-307.

Group-C: *vigorous*

Pink coloured profuse conidial growth which reaches upto the plug. The following 11 mutants fall in this group.

VgT₄-27, *VgT₄*-55, *VgT₃*-79, *VgT₂*-99, *VgT₄*-109, *VgT₃*-147, *VgT₄*-179, *VgT₃*-213, *VgT₄*-251, *VgT₄*-295, *VgT₄*-313.

Group-D: *conidial band*

Dense conidial growth from a band shaped structure at the top. colour of the conidia is pink or orange. This group include the following 9 mutants.

Con-band T₄-17, *Con-band T₃*-38, *Con-band T₂*-63, *Con-band T₄*-88, *Con-band T₃*-123, *Con-band T₄*-149, *Con-band T₄*-189, *Con-band T₄*-227, *Con-band T₃*-300.

Group-E: *fluffy*

Mycelia are colourless conidia are absent of very few in number, mycelia are cottony and growth is vigorous. The following 8 mutants fall into this groups.

flT₄-470, flT₃-319, flT₃-339, flT₃-379, flT₄-433, flT₂-286, flT₃-328, flT₃-317.

Group-F: *checked*.

Growth less than wild, conidia, light pink in colouration. The following 7 mutants fall into this group.

ChT₃-321, ChT₃-351, ChT₄-467, ChT₂-205, ChT₄-431, ChT₄-499, ChT₃-317.

Group-G: *mat*

Growth lie on the media, mycelia colourless, conidia light pink or colourless the growth cannot be easily separated from media, often conidia absent.

This group include the following 8 mutants.

matT₃-352, matT₄-482, matT₂-200, matT₁-120, matT₃-341, matT₄-491, matT₃-326, matT₃-392.

Group-H: *cauliflower*

Checked growth, conidia forming *cauliflower* like structure. The following 6 mutants fall into this group,

ClfT₃-353, ClfT₄-487, ClfT₃-343, ClfT₂-289, ClfT₂-224, ClfT₃-353.

Group-I: *dirty*.

Conidial lump scattered thought the body of culture tube. The following 5 mutants fall into this group.

dirT₄-479, dirT₃-381, dirT₄-461, dirT₃-218, dirT₃-328.

Table-5

Classification and Nomenclature of Tulsh (*Ocimum sanctum* Linn.). induced mutants.

Name of the Group	Characteristics	Number of mutant	Name of the mutant
A	Mycelia from rope like structure & conidia pink	12	<i>ropy</i>
B	Both mycelia and conidia are colourless.	10	<i>albino</i>
C	Profuse conidial and mycelial growth reach the plug.	11	<i>vigorous</i>
D	Conidia form a nice band at the tip of the growth.	9	<i>conidial band</i>
E	Profuse growth of cottony mycelia, less number of conidia.	8	<i>fluffy</i>
F	Checked growth, mycelia colourless, conidia light pink.	7	<i>checked</i>
G	Growth lie on the media, mycelia colourless, conidia light pink or colourless, the growth can not be easily separated from media, often conidia absent.	8	<i>mat</i>
H	Checked growth, conidia forming cauliflower like structure.	6	<i>cauliflower</i>
I	Conidial lump scattered throughout the body of culture tube.	5	<i>dirty</i>



Fig-5: Wild type Em'a' and some morphological mutants induced with leaf extracts of *Ocimum sanctum* Linn.

3. EFFECT OF FRUIT EXTRANCT OF *Averrhoa carambola* linn. ON THE GROWTH OF *Neuraspora crassa*.



Fig-6: Photograph of a medicinal plant kamranga (*Averrhoa carambola* Linn.).

Different concentrations of kamranga solutions were used to test the mutagenic effect on *Neuraspora crassa*.

- (a) Preparation of different extracts of kamranga (*Averrhoa carambola* Linn.). *Fruits*.

Different concentration of fruit extract of kamranga solutions with sterilized distilled water were prepared.

Table-6

Different concentrations of kamranga (*Averrhoa carambola* Linn.) extract solution.

Name of the solutions	Amount of crude extracts	Amount of sterilized distilled water	Concentration of solution in %
K _p	10ml pure fruit extracts	0	100% crud extract
K ₁	10ml crude extracts	10ml sterilized distilled water	50% crud extract
K ₂	10 l crude extracts	20ml sterilized distilled water	33.33% crud extract
K ₃	10ml crude extracts	30ml sterilized distilled water	25% crud extract
K ₄	10ml crude extracts	40ml sterilized distilled water	20% crud extract

The prepared solutions were kept separately in sterilized test tubes.

(b). Effect of fruit extracts of *Averrhoa carambola* Linn. (kamranga), on the growth of *N. crassa*.

For testing the effect, 1 ml of different solutions were taken separately on the sterilized petridishes with the help of sterilized pipette. The petridishes were marked as Op (100% crude extract), O₁, (50% extract), O₃ (25% extract), O₄ (20% extract) and O₀ (no extract). 10 ml of molten VM was added in each petridish. When media became solid Ema was inoculated at the centre of the media. Plates were incubated at 25°C and measured the radial growth after 18, 20, 22, 24 and 40 hours. Following were the results.

Table-7

Radial growth of Ema on VM containing Kamranga (*Averrhoa carambola* Linn.) fruit extracts.

Plate No. and concentration of the solution	Observation after the following hours growth in mm.									
	18 hours		20 hours		24 hours		40 hours		44 hours	
	Growth in mm.	Mean in mm.	Growth in mm.	Mean in mm.	Growth in mm.	Mean in mm.	Growth in mm.	Mean in mm.	Growth in mm.	Mean in mm.
Op (100% crude extract)	1		1.5		2		2.5		5	
		1		1.5		2		2.5		5
O ₁ (50% crude extract)	3		4		5		6		14	
		3.5		4.5		5.5		6.5		15
O ₃ (25% crude extract)	7		9		11		13		29	
		7.5		9.58		11.5		13.5		29.5
O ₄ (20% crude extract)	9		12		15		18		35	
		9.5		12.5		15.5		18.5		36
O ₀ (No extract, only VM)	29		32		36		40		over	
		29.5		33		36.5		41		over
	30		34		37		42		over	

Extracts of Kamranga (*Averrhoa carambola* Linn.) fruit showed antifungal properties, So it restricted the growth of *Neurospora crassa*.

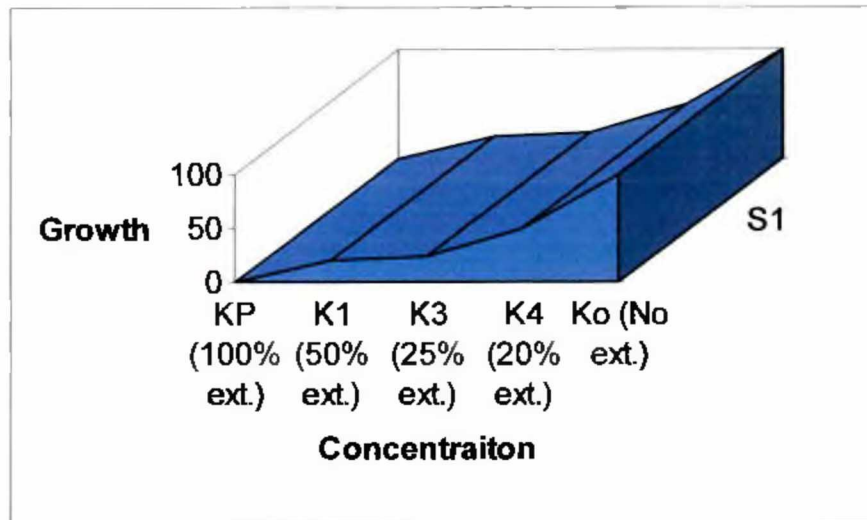


Fig:7-Bar graph of Radial growth of Ema with fruit extracts of Kamranga (*Averrhoa carambola* L.)

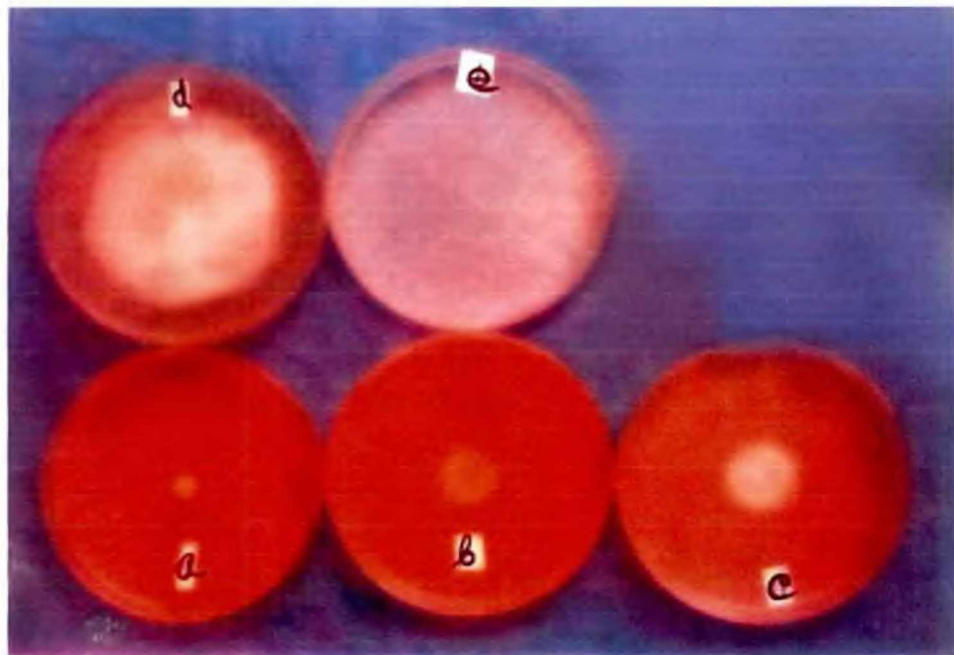


Fig-8: Comparative radial growth of Ema on VM containing fruit extracts of Kamranga (*Averrhoa carambola* Linn.).

C). Treating of Ema with extracts of fruit *Averrhoa carambola* Linn. (Kamranga) fruit :

Pure (100%) fruit extracts of *Averrhoa carambola* Linn. (Kamranga) were chosen for treating Ema. The treating time were 1 hours, 2 hours, 4 hours and 6 hours previously.

Classification of Kamranga induced mutant:

Group-A: *ropy*:

Growth is checked. The hyphae are colourless and look like *ropes*, *conidia orange* and less in number 14 mutants of this group are as follows:

roK₃-367, roK₂-293, roK₄-416, roK₄-473, roK₄-487, roK₃-312, roK₃-332, roK₄-483, roK₂-297, roK₄-409, roK₄-437, roK₄-486, roK₃-399, roK₄-432.

Group-B: *albino*

Both mycelia and conidia are colourless. The following 11 mutants fall into this group.

alK₄-416, alK₄-433, alK₃-368, alK₂-278, alK₄-400, alK₃-321, alK₄-464, alK₄-437, alK₄-491, alK₃-338, alK₃-382.

Group-C: *plug*:

Pink coloured formed outside the tube, around the plug. The following 6 mutants fall in this group.

PlK₄-401, PlK₃-361, PlK₂-297, PlK₄-428, PlK₄-489, PlK₂-238.

Group-D: *vigorous*:

Profuse conidial and mycelial growth reach the plug. This group include the following 10 mutants.

VgK₄-470, VgK₄-431, VgK₃-393, VgK₃-343, VgK₂-299, VgK₄-439, VgK₂-288, VgK₃-310, VgK₃-328, VgK₄-498.

Group-E: *conidial band*

Dense conidial growth from a band shaped structure at the top. colour of the conidia is pink or orange. This group include the following 13 mutants.

Con-band K₄-423, Con-band K₄-487, Con-band K₃-304, Con-band K₄-408, Con-band K₃-394, Con-band K₃-319,

*Con-band K₄-486, Con-band K₂-299, Con-band K₃-318,
Con-band K₃-322.*

Group-F: *fluffy*

Mycelia are colourless conidia are absent or very few in number, mycelia are cottony and growth is vigorous. The following 11 mutants fall into this groups.

*flK₄-435, flK₃-318, flK₂-268, flK₄-489, flK₃-321,
flK₁-150, flK₄-487, flK₃-328, flK₄-488, flK₃-311, flK₃-379.*

Group-G: *fluffy band*:

Colourless mycelia form a band shaped structure at the tip, steady in the tube. The following 4 mutants fall into this group.

fl-bandK₄-444, fl-bandK₃-379, fl-bandK₄-449, fl-bandK₄-411.

Group-H: *checked*.

Growth less than wild, conidia, light pink in colouration. The following 10 mutants fall into this group.

*ChK₄-449, ChK₃-366, ChK₁-183, ChK₂-208, ChK₁-129,
ChK₂-244, ChK₃-367, ChK₄-412, ChK₄-431, ChK₃-314.*

Group-I: *mat*

Growth lie on the media, mycelia colourless, conidia light pink or colourless the growth can not be easily separated from media, often conidia absent.

This group include the following 10 mutants.

*matK₄-413, matK₃-382, matK₄-468, matK₂-292,
matK₁-113, matK₄-449, matK₄-495, matK₃-342, matK₄-481, matK₃-342.*

Group-J: *yeallow fluffy*:

Conidia less, mycelia yellow. Mycelia form a band like structure at the tip of the culture tube. The following 3 mutants fall into this group,

Y-flK₄-412, Y-flK₃-327, Y-flK₄-478.

Group-K: cauliflower

Checked growth, conidia forming cauliflower like structure. The following 5 mutants fall into this group,

ClfK₃-311, ClfK₄-418, ClfK₃-381, ClfK₃-302, ClfK₄-453.

Group-L: orange :

Deep orange conidia. The following 2 mutants fall into this group.

OrK₄-433, OrK₄-412.

Table-8

Classification and Nomenclature of Kamranga (*Averrhoa camrambola* Linn.). induced mutants.

Name of the Group	Characteristics	Number of mutant	Name of the mutant
A	Mycelia from rope like structure & conidia pink	14	<i>rope</i>
B	Both mycelia and conidia are colourless.	11	<i>albino</i>
C	Pink conidia formed outside the tube, around the plug.	6	<i>plug</i>
D	Profuse conidial and mycelial growth reach the plug.	10	<i>vigorous</i>
E	Conidia form a nice band at the tip of the growth.	13	<i>conidial band</i>
F	Profuse growth of cottony mycelia, less number of conidia.	11	<i>fluffy</i>
G	Colourless mycelia form a band shaped structure at the tip, steady in the tube.	4	<i>fluffy band</i>
H	Checked growth, mycelia colourless, conidia light pink.	10	<i>checked</i>
I	Growth lie on the media, mycelia colourless, conidia light pink or colourless, the growth can not be easily separated from media, often conidia absent.	10	<i>mat</i>
J	Conidia less, mycelia yellow. Mycelia form a band like structure at the tip of the culture tube.	3	<i>yellow fluffy</i>
K	Checked growth, conidia forming cauliflower like structure.	5	<i>Cauliflower</i>
L	Deep orange conidia.	2	<i>Orange</i>



Fig-9: Wild type Em'a' and some morphological mutants induced with fruit extracts of *Averrhoa carambola* Linn.

4. EFFECT OF LEAF EXTRACT OF SHETADRON (*Leucas lavandulaefolia* Rees.) ON THE GROWTH OF *Neuraspota crassa*.



Fig-10: Photograph of a medicinal plant *Leucas lavandulaefolia* Rees. (Shetadron).

Different concentrations of Shetadron solutions were used to test the mutagenic effect on *Neuraspota crassa*.

- (a) Preparation of different extracts of Shetadron (*Leucas lavandulaefolia* Rees.) leaves.

Different concentration of fruit extract of Shetadon solutions with sterilized distilled water were prepared.

Table-9

Different concentrations of Shetadron (*Leucas lavandulaefolia* Rees.) extract solution.

Name of the solutions	Amount of crude extracts	Amount of sterilized distilled water	Concentration of solution in %
S _p	10 ml pure leaves extracts	0	100% crude extract
S ₁	10 ml crude extracts	10 ml sterilized distilled water	50% crude extract
S ₂	10 ml crude extracts	20 ml sterilized distilled water	33.33% crude extract
S ₃	10 ml crude extracts	30 ml sterilized distilled water	25% crude extract
S ₄	10 ml crude extracts	40 ml sterilized distilled water	20% crude extract

The prepared solutions were kept separately in sterilized test tubes.

- (b). Effect of leaf extracts of *Leucas bavandulaefolia* Rees. (Shetadron on the growth of *N. crassa*.

For testing the effect, 1 ml of different solutions were taken separately on the sterilized petridishes with the help of sterilized pipette. The petridishes were marked as Op (100% crude extract), O₁, (50% extract), O₃ (25% extract) and O₀ (no extract). 10 ml of molten VM was added in each petridish. When media became solid Ema was inoculated at the centre of the media. Plates were incubated at 25°C and measured the radial growth after 18, 20, 24 and 40 hours. Following were the results.

Extracts of *Leucas lavandulaefolia* Rees. (Shetadron) leaf showed antifungal properties. So it restricted the growth of *Neurospora crassa*.

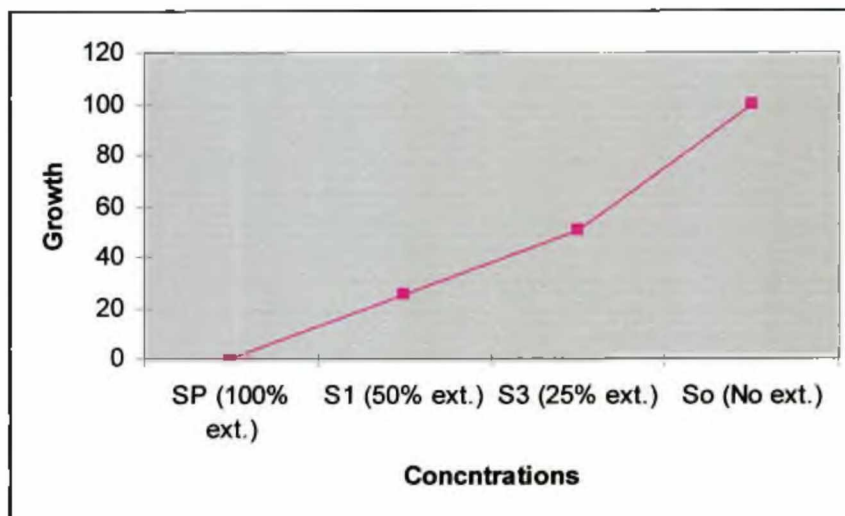


Fig:-11. Graph of Radial growth of Ema with the leaf extracts of *Leucas lavandulaefolia* Rees. (Shetadron).



Fig-12: Comparative radial growth of Ema on VM containing leaf extracts of Shetadron (*Leucas lavandulaefolia* Rees.).

C). Treating of Ema with extracts of *Leucas lavandulaefolia* Rees. (Shetadron) Leaf:

Pure (100%) leaf extracts of Shetadron (*Leucas lavandulaefolia* Rees.) were chosen for treating Ema. The treating time were 1 hours, 2 hours, 4 hours and 6 hours previously.

Classification of Shetadron induced mutant :

Group-A: ropy:

Growth is checked. The hyphae are colourless and look like ropes, conidia orange and less in number 11 mutants of this group are as follows:

roS₃-302, roS₄-409, roS₂-215, roS₁-124, roS₄-428, roS₃-329, roS₃-353, roS₄-458, roS₃-373, roS₄-452, roS₁-105.

Group-B: plug:

Pink coloured formed outside the tube, around the plug. The following 5 mutants fall in this group.

plS₁-79, plS₂-249, plS₃-311, plS₄-413, plS₄-432.

Group-C: vigorous:

Profuse conidial and mycelial growth reach the plug. This group include the following 10 mutants.

vgS₁-68, vgS₁-77, vgS₁-176, vgS₂-203, vgS₂-234, vgS₃-313, vgS₃-341, vgS₃-368, vgS₃-372, vgS₄-416.

Group-D: albino

Both mycelia and conidia are colourless. The following 10 mutants fall into this group.

alS₁-62, alS₂-217, alS₂-258, alS₂-292, alS₃-308, alS₃-319, alS₃-339, alS₃-376, alS₄-418, alS₄-451.

Group-E: conidial band :

Dense conidial growth from a band shaped structure at the top. colour of the conidia is pink or orange. This group include the following 11 mutants.

con-band S₁-43, con-band S₁-97, con-band S₂-211, con-band S₂-223, con-band S₂-259, con-band S₂-279, con-band S₃-319, con-band S₃-361, con-band S₃-391, con-band S₄-423, con-band S₄-473.

Group-F: *fluffy*:

Mycelia are colourless conidia are absent or very few in number, mycelia are cottony and growth is vigorous. The following 9 mutants fall into this groups.

*flS*₁-51, *flS*₂-207, *flS*₂-221, *flS*₂-273, *flS*₃-318, *flS*₃-331, *flS*₃-333, *flS*₄-433, *flS*₄-494.

Group-G: *checked* :

Growth less than wild, conidia, light pink in colouration. The following 10 mutants fall into this group.

*chS*₁-33, *chS*₁-149, *chS*₂-213, *chS*₂-298, *chS*₃-311, *chS*₃-329, *chS*₃-371, *chS*₄-408, *chS*₄-429, *chS*₄-483.

Group-H: *mat* :

Growth lie on the media, mycelia colourless, conidia light pink or colourless the growth cannot be easily separated from media, often conidia absent.

This group include the following 9 mutants.

*matS*₁-155, *matS*₂-232, *matS*₂-283, *matS*₃-318, *matS*₃-333, *matS*₃-364, *matS*₄-411, *matS*₄-426, *matS*₄-498.

Group-I: *cauliflower*:

Checked growth, conidia forming cauliflower like structure. The following 4 mutants fall into this group,

*clfS*₃-334, *clfS*₃-344, *clfS*₄-438, *clfS*₄-475.

Group-J: *fluffy band* :

Colourless mycelia form a band shaped structure at the tip, steady in the tube. The following 5 mutants fall into this group.

*fl-bandS*₃-306, *fl-bandS*₃-396, *fl-bandS*₄-425, *fl-bandS*₄-447, *fl-bandS*₄-482.

Group-K: *dirty*:

Conidial lump scattered throughout the body of culture tube. The following 2 mutants fall into this group,

*dirS*₃-351, *dirS*₄-481.

Table-11

Classification and Nomenclature of *Shetadron (Leucas lavandulaefolia* Ress.) induced mutants.

Name of the Group	Characteristics	Number of mutant	Name of the mutant
A	Mycelia from rope like structure & conidia pink	11	<i>ropy</i>
B	Pink conidia formed outside the tube, around the plug.	5	<i>plug</i>
C	Profuse conidial and mycelial growth reach the plug.	10	<i>vigorous</i>
D	Both mycelia and conidia are colourless.	10	<i>albino</i>
E	Conidia form a nice band at the tip of the growth.	11	<i>conidial band</i>
F	Profuse growth of cottony mycelia, less number of conidia.	9	<i>fluffy</i>
G	Checked growth, mycelia colourless, conidia light pink.	10	<i>checked</i>
H	Growth lie on the media, mycelia colourless, conidia light pink or colourless, the growth can not be easily separated from media, often conidia absent.	9	<i>mat</i>
I	Checked growth, conidia forming cauliflower like structure.	4	<i>cauliflower</i>
J	Clourless mycelia form a band shaped structure at the tip, steady in the tube.	5	<i>fluffy band</i>
K	Conidial lump scattered throughout the body of culture tube.	2	<i>dirty</i>



Fig-13: Wild type Em`a' and some morphological mutants induced with leaf extracts of Shetadron (*Leucas lavandulaefolia* Ress.)

5. EFFECT OF LEAF EXTRANCT OF *Coccinea cordifolia* Linn. (TELAKUCHA) ON THE GROWTH OF *Neuraspora crassa*.



Fig-14: Photograph of a medicinal plant *Coccinea cordifolia* Linn. (Telakucha).

Different concentrations of Telakucha solutions were used to test the mutagenic effect on *Neuraspora crassa*.

- (a) Preparation of different extracts of *Coccinea cordifolia* Linn. (Telakucha) leaves.

Different concentration of leaf extract of Telakucha solutions with sterilized distilled water were prepared.

Table-12

Different concentrations of *Coccinea cordifolia* Linn. (Telakucha) extract solution.

Name of the solutions	Amount of crude extracts	Amount of sterilized distilled water	Concentration of solution in %
C _P	10ml pure leves extracts	0	100% crud extract
C ₁	10ml crude extracts	10ml sterilized distilled water	50% crud extract
C ₂	10ml crude extracts	20ml sterilized distilled water	33.33% crud extract
C ₃	10ml crude extracts	30ml sterilized distilled water	25% crud extract
C ₄	10ml crude extracts	40ml sterilized distilled water	20% crud extract

The prepared solutions were kept separately in sterilized test tubes.

(b). Effect of leaf extracts of *Coccinea cordifolia* Linn. (Telakucha) on the growth of *N. crassa*.

For testing the effect, 1 ml of different solutions were taken separately on the sterilized petridishes with the help of sterilized pipette. The petridishes were marked as Op (100% crude extract), O₁, (50% extract), O₃ (25% extract) and O₀ (no extract). 10 ml of molten VM was added in each petridish. When media became solid Ema was inoculated at the centre of the media. Plates were incubated at 25°C and measured the radial growth after 18, 20, 24 and 40 hours. Following were the results.

Table-13

Radial growth of Ema on VM containing Telakucha (*Coccinea cordifolia* Linn.) leaf extracts.

Plate No. and concentration of the solution	Observation after the following hours growth in mm.							
	18 hours		20 hours		24 hours		40 hours	
	Growth in mm.	Mean in mm.	Growth in mm.	Mean in mm.	Growth in mm.	Mean in mm.	Growth in mm.	Mean in mm.
Op (100% crude extract)	6	6.5	8	8.5	11	11.5	26	26.5
	7		9		12		27	
O ₁ (50% crude extract)	10	10.5	13	13.5	16	16.5	32	33
	11		14		17		34	
O ₃ (25% crude extract)	17	18	20	21	25	26	44	45
	19		22		27		46	
O ₀ (No extract, only VM)	25	25.5	28	28.5	34	34.5	over	over
	26		29		35		over	

Extracts of *Coccinea cordifolia* Linn. (Telakucha) leaf showed antifungal properties. So it restricted the growth of *Neurospora crassa*.

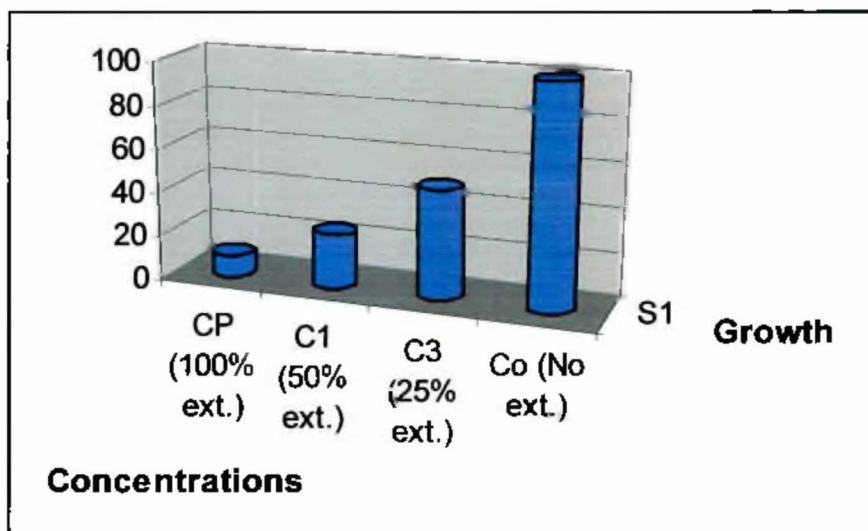


Fig:15. Bar graph of Radial growth of Ema with the leaf extracts of Telakucha (*Coccinea cordifolia* Linn.)

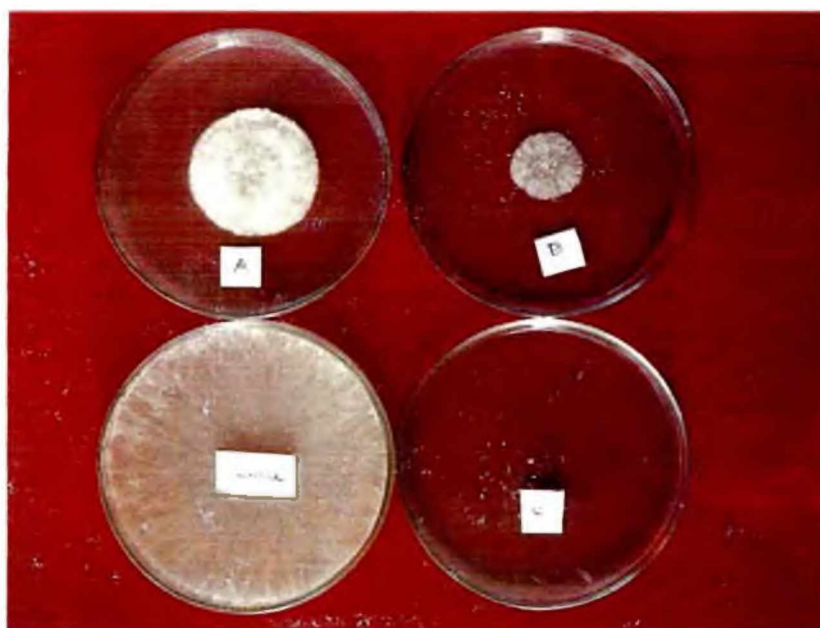


Fig:16: Comparative radial growth of Ema on VM containing leaf extracts of Telakucha (*Coccinea cordifolia* Linn.)

c). Treating of Ema with extracts of *Coccinea cordifolia* Linn. (Telakucha).

Pure (100%) leaf extracts of Telakucha (*Coccinea cordifolia* Linn.) were chosen for treating Ema. The treating time were 1 hours, 2 hours, 4 hours and 6 hours previously.

Classification of Telakucha induced mutant :

Group-A: *albino*

Both mycelia and conidia are colourless. The following 10 mutants fall into this group.

alC₁-16, alC₁-78, alC₁-125, alC₂-262, alC₂-278, alC₂-298, alC₃-309, alC₃-323, alC₃-373, alC₄-432.

Group-B: *vigorous*:

Profuse conidial and mycelial growth reach the plug. This group include the following 10 mutants.

vgC₁-18, vgC₁-60, vgC₁-132, vgC₂-212, vgC₂-226, vgC₂-268, vgC₃-310, vgC₃-324, vgC₄-420, vgC₄-448.

Group-C: *ropy*:

Growth is checked. The hyphae are colourless and look like ropes, conidia orange and less in number 12 mutants of this group are as follows:

roC₁-17, roC₁-97, roC₁-163, roC₁-198, roC₂-213, roC₂-242, roC₂-278, roC₃-307, roC₃-341, roC₃-374, roC₄-413, roC₄-494.

Group-D: *plug*:

Pink coloured formed outside the tube, around the plug. The following 4 mutants fall in this group.

plC₃-312, plC₄-414, plC₄-427, plC₄-490.

Group-E: *fluffy*:

Mycelia are colourless conidia are absent or very few in number, mycelia are cottony and growth is vigorous. The following 9 mutants fall into this groups.

*flC*₁-51, *flC*₂-207, *flC*₂-222, *flC*₂-289, *flC*₃-318, *flC*₃-333, *flC*₃-366, *flS*₄-444, *flS*₄-494.

Group-F: *mat* :

Growth lie on the media, mycelia colourless, conidia light pink or colourless the growth can not be easily separated from media, often conidia absent.

This group include the following 8 mutants.

*matC*₁-157, *matC*₂-232, *matC*₂-283, *matC*₃-318, *matC*₃-338, *matC*₃-364, *matC*₄-411, *matC*₄-493.

Group-G : *conidial band* :

Dense conidial growth from a band shaped structure at the tip, colour of the conidia is pink or orange. This group include the following 10 mutants.

*con-band C*₁-43, *con-band C*₁-97, *con-band C*₂-211, *con-band C*₂-223, *con-band C*₂-259, *con-band C*₂-279, *con-band C*₃-319, *con-band C*₃-361, *con-band C*₃-391, *con-band C*₄-423.

Group-H : *cauliflower*:

Checked growth, conidia forming cauliflower like structure. The following 3 mutants fall into this group,

*clfC*₃-344, *clfC*₄-438, *clfC*₄-475.

Group-I : *checked* :

Growth less than wild, conidia light pink in colouration. The following 9 mutants fall into this group.

*chC*₁-33, *chC*₁-149, *chC*₂-213, *chC*₂-298, *chC*₃-311, *chC*₃-329, *chC*₄-408, *chC*₄-429, *chC*₄-483.

Group-J : fluffy band :

Colourless mycelia form a band shaped structure at the tip, steady in the tube. The following 7 mutants fall into this group.

fl-bandC₃-307, *fl-bandC₃-396*, *fl-bandC₄-425*, *fl-bandC₄-447*, *fl-bandC₄-452*, *fl-bandC₄-463*, *fl-bandC₄-483*.

Table-14

Classification and Nomenclature of Telakucha (*Coccinea cordifolia* Linn.) induced mutants.

Name of the Group	Characteristics	Number of mutant	Name of the mutant
A	Both mycelia and conidia are colourless.	10	<i>albino</i>
B	Profuse conidial and mycelial growth reach the plug.	10	<i>vigorous</i>
C	Mycelia form rope like structure & conidia pink	12	<i>ropy</i>
D	Pink conidia formed outside the tube, around the plug.	4	<i>plug</i>
E	Profuse growth of cottony mycelia, less number of conidia.	9	<i>fluffy</i>
F	Growth lie on the media, mycelia colourless, conidia light pink or colourless, the growth can not be easily separated from media, often conidia absent.	8	<i>mat</i>
G	Conidia form a nice band at the tip of the growth.	10	<i>conidial band</i>
H	Checked growth, conidia forming cauliflower like structure.	3	<i>cauliflower</i>
I	Checked growth, mycelia colourless, conidia light pink.	9	<i>checked</i>
J	Colourless mycelia form a band shaped structure at the tip, steady in the tube.	5	<i>fluffy band</i>

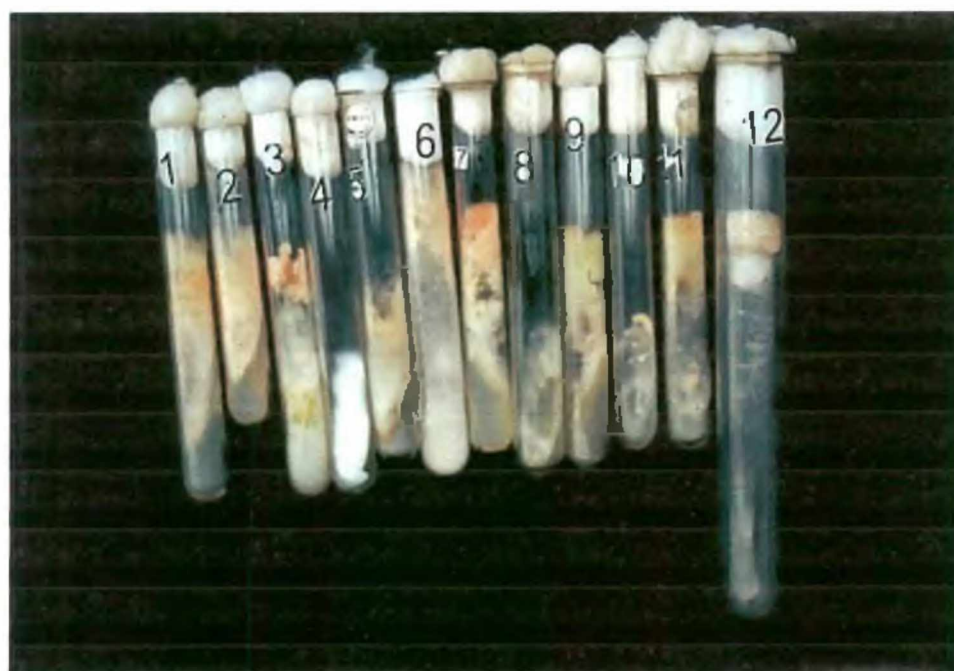


Fig-17: Wild type Em'a' and some morphological mutants induced with leaf extracts of Telakucha (*Coccinea cordifolia* Linn.)

6. INDUCTION OF MUTATION BY UV IRRADIATION.

UV light of 254nm wave length was used for treating conidia and the treatment was done in the dark to avoid photo reactivating. The conidia of Ema were treated for 2, 2½ and 3 minutes at the distance of 15cm. SM⁺ leucine, lysine, adenine, arginine, histidine and tryptophan was used for plating and VM with supplements were used for isolating conidial colonies. Single colonies were isolated and incubated at 25°C. After 5 days the cultures that showed changes in its morphology comparing with Ema of the same age were taken as the morphological mutants. The mutants were different from Ema in colouration of conidia, mode of conidiation and mycelial growth etc.

The mutants were compared with the wild type Ema and classified.

Classification of UV induced mutants :

Group-A: *vigorous*:

Profuse conidial and mycelial growth reach the plug. This group include the following 7 mutants.

vgU-104, vgU-127, vgU-185, vgU-212, vgU-289, vgU-311, vgU-370.

Group-B: *ropy*:

Growth is checked. The hyphae are colourless and look like ropes, conidia orange and less in number 9 mutants of this group are as follows:

roU-36, roU-57, roU-92, roU-107, roU-120, roU-193, roU-216, roU-273, roU-314.

Group-C: *albino*

Both mycelia and conidia are colourless. The following 7 mutants fall into this group.

alU-69, alU-97, alU-118, alU-183, alU-205, alU-295, alU-317.

Group-D: *crescent* :

Conidial and mycelial growth form a nice crescent at the tip of the growth, mycelia colourless and steady in the tube, conidia light pink. The following 4 mutants fall in this group.

crsU-88, crsU-196, crsU-248, crsU-376.

Group-E: *mat* :

Growth lie on the media, mycelia colourless, conidia light pink or colourless the growth cannot be easily separated from media, often conidia absent.

This group include the following 6 mutants.

matU-76, matU-189, matU-219, matU-287, matU-326, matU-432.

Group-F : *checked* :

Growth less than wild, conidia, light pink in colouration. The following 8 mutants fall into this group.

chU-27, chU-57, chU-89, chU-103, chU-133, chU-240, chU-332, chU-392.

Group-G: *plug*:

Pink coloured formed outside the tube, around the plug. The following 4 mutants fall in this group.

plU- 78, plU-138, plU-202, plU-327.

Group-H: *fluffy*:

Mycelia are colourless conidia are absent of very few in number, mycelia are cottony and growth is vigorous. The following 9 mutants fall into this groups.

flU-10, flU-33, flU-83, flU-143, flU-174, flU-219, flU-299, flU-321, flU-384.

Group-I : *conidial band* :

Dense conidial growth from a band shaped structure at the tip, colour of the conidia is pink or orange. This group include the following 8 mutants.

con-band U-2, con-band U-31, con-band U-91, con-band U-136, con-band U-196, con-band U-226, con-band U-277, con-band U-333.

Group-J : *orange*:

Deep orange conidia.

orgU-170 fall under this group.

Group-K : *fluffy band* :

Colourless mycelia form a band shaped structure at the tip, steady in the tube. The following 5 mutants fall into this group.

fl-bandU-66, fl-bandU-144, fl-bandU-231, fl-bandU-299, fl-bandU-381.

Group-L : *cauliflower*:

Checked growth, conidia forming cauliflower like structure. The following 2 mutants fall into this group,

clfU-256, clfU-338.

Group-M: *dirty*:

Conidial lump scattered throughout the body of culture tube.

dirU-171 fall under this group.

Table-15

Classification and Nomenclature of UV induced mutants.

Name of the Group	Characteristics	Number of mutant	Name of the mutant
A	Profuse conidial and mycelial growth reach the plug.	7	<i>vigorous</i>
B	Mycelia from rope like structure & conidia pink	9	<i>ropy</i>
C	Both mycelia and conidia are colourless.	7	<i>albino</i>
D	Conidial and mycelial growth form a nice crescent at the tip of the growth mycelia colourless and steady in the tube, conidia light pink.	4	<i>crescent</i>
E	Growth lie on the media, mycelia colourless, conidia light pink or colourless, the growth can not be easily separated from media, often conidia absent.	6	<i>mat</i>
F	Checked growth, mycelia colourless, conidia light pink.	8	<i>checked</i>
G	Pink conidia formed outside the tube, around the plug.	4	<i>plug</i>
H	Profuse growth of cottony mycelia, less number of conidia.	9	<i>fluffy</i>
I	Conidia form a nice band at the tip of the growth.	8	<i>conidial band</i>
J	Deep orange conidia.	1	<i>orange</i>
K	Colourless mycelia form a band shaped structure at the tip, steady in the tube.	5	<i>fluffy band</i>
L	Checked growth, conidia forming cauliflower like structure.	2	<i>cauliflower</i>
M	Conidial lump scattered throughout the body of culture tube.	1	<i>dirty</i>



Fig-18: Wild type Em'a' and some morphological mutants induced with UV irradiation.

**DETAILED STUDY OF SOME LEAF
EXTRACT OF *Ocimum sanctum* Linn.
Leucas lavandulaefolia Ress,
Coccinea cordifolia Linn.,
FRUIT EXTRACT
OF
Averrhoa carambola Linn.
AND
UV IRRADIATION INDUCED MUTANTS.**

Detailed study of some selected mutants induced with leaf extract of *Ocimum sanctum* Linn, *Leucas, lavandulaefolia* Rees., *Coccinea cordifolia* Linn. , fruit extract of *Averrhoa carambola* Linn. and UV irradiation induced mutants.

Table -16

The following 14 mutants obtained from leaf extract of *Ocimum Sanctum* Linn, *Leucas lavaendulaefolia* Ress., *Coccinea Cordifolia* Linn., fruit extract of *Averrhoa carambola* Linn. and UV irradiation were selected for further analysis.

No of the Mutant	Name of the mutant
K ₄ 409	<i>ropy</i>
K ₄ 470	<i>vigorous</i>
T ₃ 317	<i>checked</i>
K ₄ 401	<i>plug</i>
S ₃ 308	<i>albino</i>
S ₄ 411	<i>mat</i>
C ₃ 366	<i>fluffy</i>
C ₄ 452	<i>fluff-bard</i>
T ₄ 461	<i>dirty</i>
K ₂ 299	<i>conidial-band</i>
K ₄ 412	<i>orange</i>
U256	<i>cauliflower</i>
U376	<i>crescent</i>
K ₄ 478	<i>yellow fluffy-band</i>



Fig-:19The wild type Ema (5297)



Fig-20 : The wild type EmA (5296)



Fig-21: The morphological mutant *ropy*.

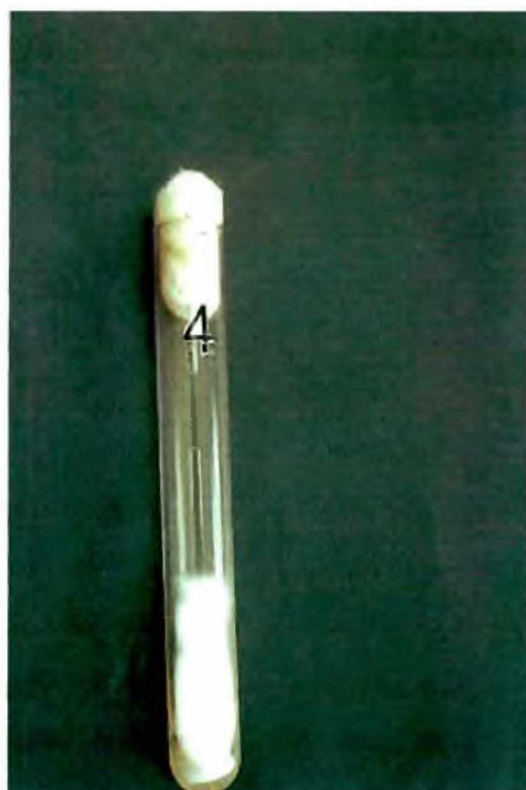


Fig-22 : The morphological mutant *albino*.

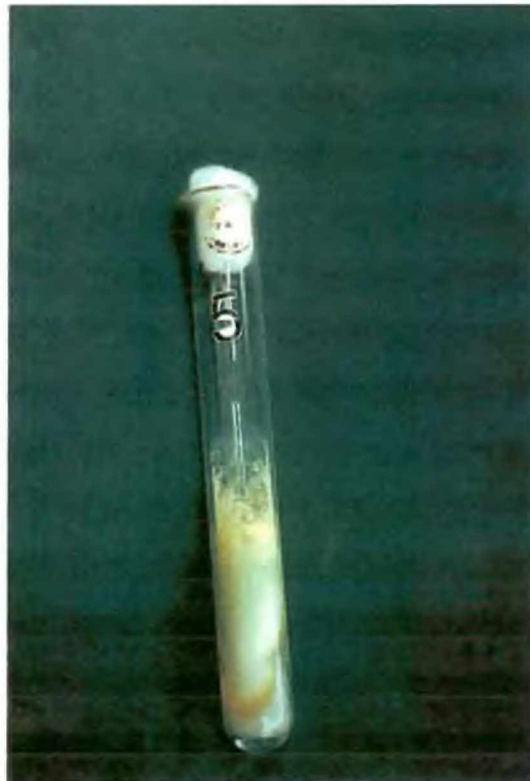


Fig-23 : The morphological mutant *check*.

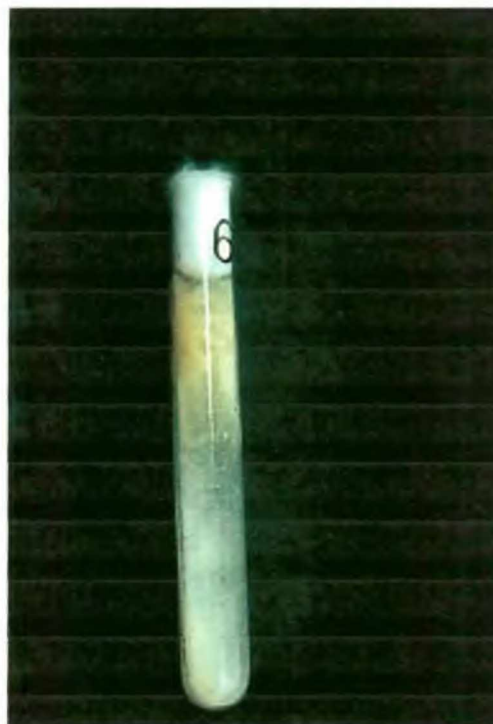


Fig-24 : The morphological mutant *vigorous* .



Fig- 25 : The morphological mutant *cauliflower*



Fig- 26 : The morphological mutant *mat*



Fig- 27: The morphological mutant *fluffy*



Fig- 28 : The morphological mutant *crescent*



Fig- 29 : The morphological mutant *conidial-band*.



Fig- 30 : The morphological mutant *plug*



Fig- 31 : The morphological mutant *yellow fluffy*



Fig- 32 : The morphological mutant *fluffy band*



Fig- 33 : The morphological mutant *dirty*.



Fig- 34 : The morphological mutant *buff*

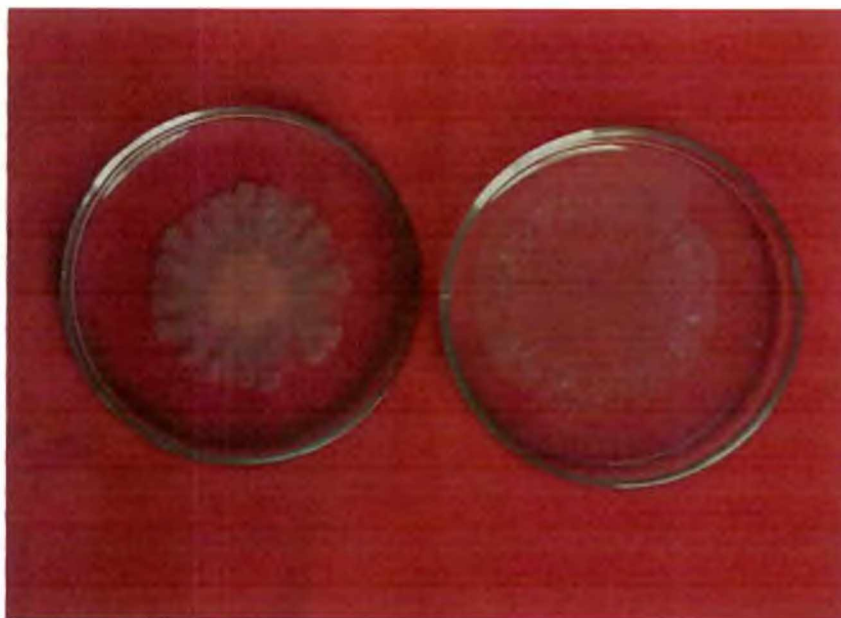


Fig-35. Colonial features of *ropy* and *fluffy-band* (left to right)

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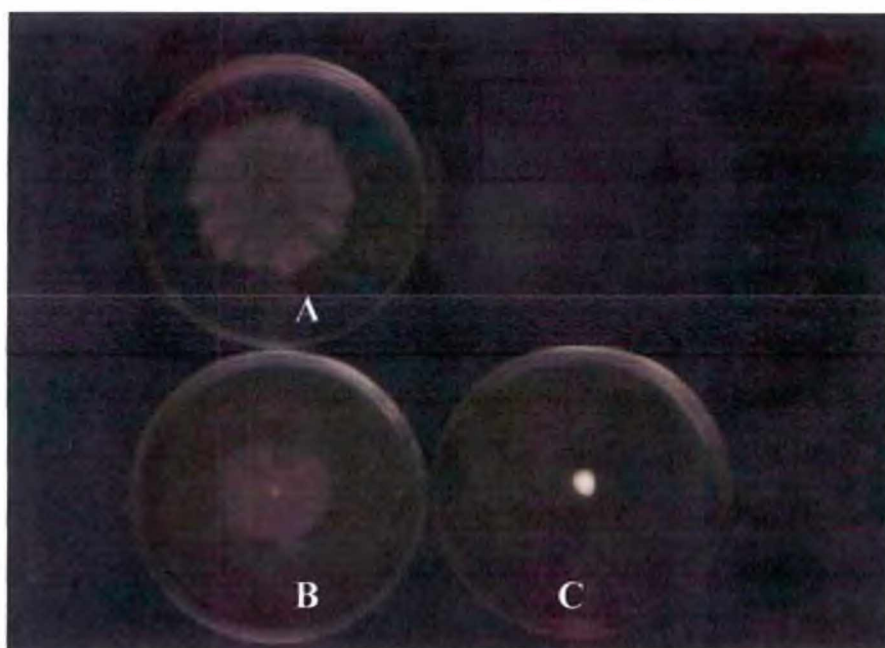


Fig. 36: Colonial features of Em 'a' (5297), *crescent* and *orange* (Left to right)

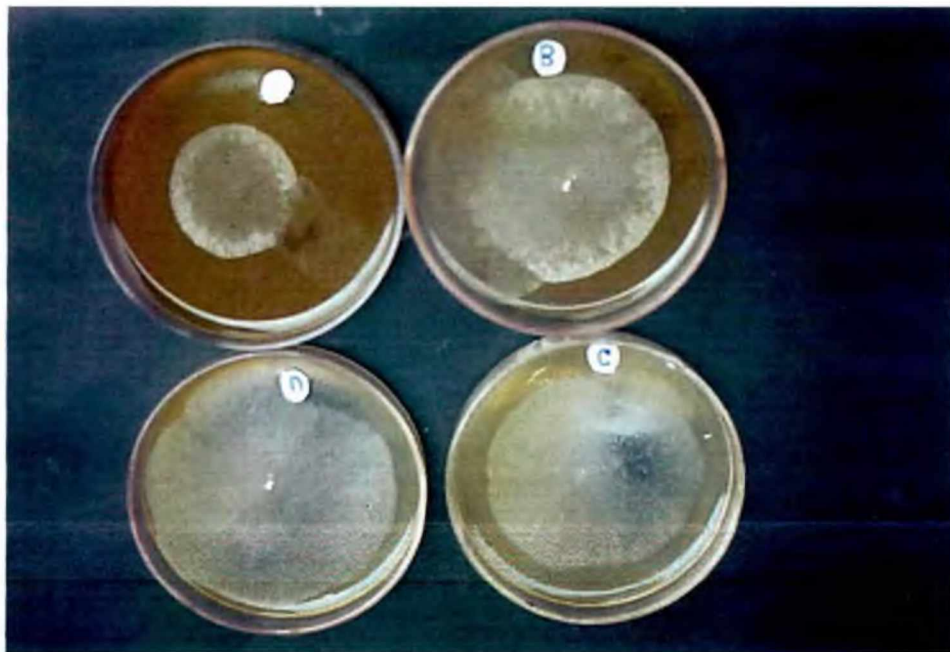


Fig.37: Colonial features of E m 'a' (5297) *fluffy* and *con. band*.

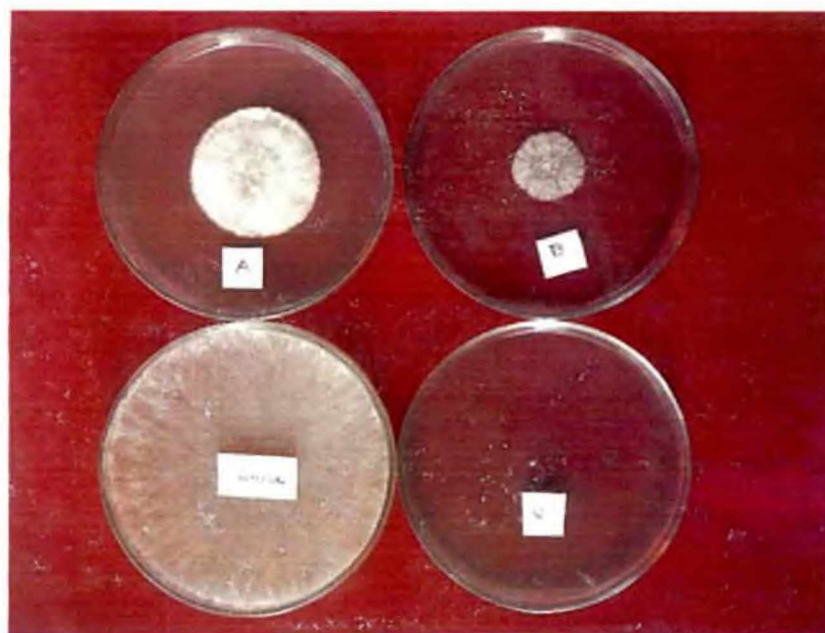


Fig-38, Colnical features of Em 'a', *ropy*, *check* and *albino*.

COMPLEMENTATION TESTS ON SOLID AND IN LIQUID MEDIA OF THE SELECTED MORPHOLOGICAL MUTANTS.

The selected morphological mutants were subcultured 3 times to obtain fresh cultures. Small tubes were used for preparation of VM medium. Observation were taken after, 3, 5, 7, 15, and 21 days.

For further confirmation, they are tested in liquid media. In every case there were duplicate test with duplicate controls the results are given in the figure.

The following mutants were selected for the heterocaryon test.

vigorous (K₄-470), *conidial band* (K₂-299), *fluffy* (C₃-366), *ropy* (K₄-409), *mat* (S₄ -411) *cauliflower* (u-256) and *checked* (T₃- 317) and *fluffy band* (C₄- 452) were undertaken by complementation test.

	<i>vg</i> (470)	<i>con-band</i> (299)	<i>fl</i> (366)	<i>ro</i> (409)	<i>mat</i> (411)	<i>clf</i> (256)	<i>ch</i> (317)	<i>fl-band</i> (452)
<i>vg</i> (470)	0	•	•	•	•	•	•	•
<i>con-band</i> (299)		0	•	•	•	•	•	•
<i>l</i> (366)			0	•	•	•	•	•
<i>ro</i> (409)				0	•	•	•	•
<i>mat</i> (411)					0	•	•	•
<i>clf</i> (256)						0	•	•
<i>ch</i> (317)							0	•
<i>fl-band</i> (452)								0

Fig:- 39. Complementation matrix of some selected mutants in solid VM media.

Note: • heterocaryon positive

0 heterocaryon negative.

	vg (470)	con-band (299)	fl(366)	ro(409)	mat(411)	clf(256)	ch(317)	fl-band (452)
vg (470)	0	•	•	•	•	•	•	•
con-band (299)		0	•	•	•	•	•	•
fl(366)			0	•	•	•	•	•
ro(409)				0	•	•	•	•
mat(411)					0	•	•	•
clf(256)						0	•	•
ch(317)							0	•
fl-band (452)								0

Fig :- 40. Complementation matrix of some selected mutants in liquid VM media.

Note: • heterocaryon positive

0 heterocaryon negative.

A) 72 hours old cultures / Mycelial weight of some selected mutants:-



Fig: - 41 . Mycelial growth of some selected mutants.

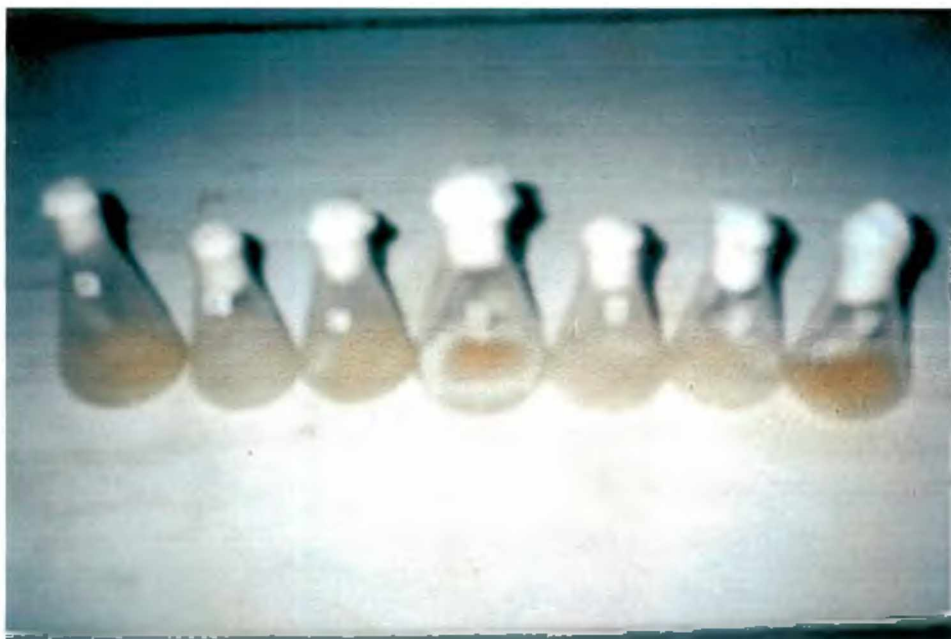


Fig: - 42 . Mycelial growth of some selected mutants.

Table-17

It was done in liquid VM medium. Selected mutants and Ema were inoculated in the medium of the flask. Each flask contained 50 ml medium, flask were shaken continuously. Weight of the empty filter papers was taken.

Name of the cultures	Weight of the empty filter paper (g.)	Weight of the filter paper with Dried mycelia (g.)	Weight of the dried mycelia (g.)	Mean (g.)
<i>ropy</i>	1.001	1.4085	0.4075	0.4124
	1.002	1.4193	0.4170	
<i>checked</i>	1.002	1.198	0.196	0.1575
	1.002	1.121	0.119	
<i>vigorous</i>	1.002	1.491	0.489	0.494
	1.002	1.501	0.499	
<i>conidial band</i>	1.003	1.5175	0.5145	0.5111
	1.002	1.5097	0.5077	
<i>fluffy</i>	1.002	1.4678	0.4658	0.466
	1.001	1.4672	0.4662	
<i>crescent</i>	1.002	1.3853	0.3833	0.39
	1.002	1.3987	0.3967	
<i>dirty</i>	1.002	1.4723	0.4703	0.4737
	1.003	1.4801	0.4771	
<i>mat</i>	1.003	1.2087	0.2057	0.19825
	1.002	1.1928	0.1908	
<i>plug</i>	1.003	1.502	0.499	0.5035
	1.003	1.511	0.508	
<i>cauliflower</i>	1.003	1.122	0.119	0.12
	1.003	1.124	0.121	
<i>albino</i>	1.002	1.4302	0.4282	0.42795
	1.002	1.4297	0.4277	
<i>fluffy band</i>	1.002	1.4689	0.4669	0.468
	1.003	1.4721	0.4691	
Ema	1.003	1.4627	0.45957	0.4599
	1.003	1.4631	0.4601	

When the culture was 72 hours old, flask with culture were boiled in water for 2 minutes and were filtered with filter paper of known weight. The filter papers with mycelia were dried in a oven and the weight of filter papers with culture were taken.

Conclusion:

It was observed in the above Table that there was variation in the dry weight of selected mutants with Ema.

- B) Germination study of some selected mutants and Ema induced by leaf extracts of *Ocimum sanctum* Linn., *Leucas lavandulaefolia* Ress., *Coccinea cordifolia* Linn, fruit extract of *Averrhoa carambola* Linn. and UV irradiation.

Table-18

Germination of some selected mutants induced by leaves & fruit extracts and UV:

Name of the Cultures	Observation of germination after following hours									
	2½ hours	3 hours	3½ hours	4 hours	4½ hours	5 hours	5½ hours	6 hours	6½ hours	7 hours
<i>plug</i>	Germination started	Germination completed								
<i>vigorous</i>	Germination not started	Germination started	Germination completed							
<i>fluffy</i>	Germination not started	Germination not started	Germination not started	Germination started	Germination completed					
<i>albino</i>	Germination not started	Germination not started	Germination not started	Conidia became swollen	Germination started	Germination completed				
<i>conidial land</i>	Germination not started	Germination not started	Germination not started	Conidia became swollen	Germination started	Germination completed				
<i>fluffy band</i>	Germination not started	Germination not started	Germination not started	Germination not started	Germination not started	Germination started	Germination completed			

Name of the Cultures	Observation of germination after following hours									
	2½ hours	3 hours	3½ hours	4 hours	4½ hours	5 hours	5½ hours	6 hours	6½ hours	7 hours
<i>cauliflower</i>	Germination not started	Germination not started	Germination not started	Germination not started	Germination not started	Conidia became swollen	Germination started	Germination completed		
<i>checked</i>	Germination not started	Germination not started	Germination not started	Germination not started	Germination not started	Conidia became swollen	Germination started	Germination completed		
<i>crescent</i>	Germination not started	Germination not started	Germination not started	Germination not started	Germination not started	Conidia became swollen	Germination started	Germination completed		
<i>ropy</i>	Germination not started	Germination not started	Germination not started	Germination not started	Germination not started	Germination not started	Germination started	Germination completed		
<i>mat</i>	Germination not started	Germination not started	Germination not started	Germination not started	Germination not started	Germination not started	Germination not started	Conidia became swollen	Germination started	Germination completed
Ema	Germination not started	Germination not started	Germination completed							

Conclusion: The mutants showed variation in there time required for germination with that of wild type Em'a'.

C) Study of linear growth:

Linear growth of Ema and some selected mutants induced with leaf extracts of *Ocimum sanctum* Linn, *Leucas lavandulaefolia* Ress, *Coccinea cordifolia* Linn, fruit extract of *Averrhoa carambola* Linn. and UV irradiation were measured in race tube, made of long Pyrex glass tube. The horizontal portion was kept 36 cm long and inner diameter of about 15mm. The two sides which were bent up one at an angle of 45° and opposite side at 90° angle with a lower projection of 1 cm. These tubes were held up right on a flat surface with a holders. Flat portion of the tube were half filled with the solid VM medium containing 4% agar. Mutants & Ema were inoculated at one end of the medium of the tube. Linear growth of the tube were measured in cm at 6 hours interval.

Linear growth of selected mutants were measured in race tube:-

Table-19
Linear growth of Ena and some selected mutants:
Linear growth in cm after the following hours

Name of the Cultures	After 24 hrs.	Mean (cm)	30 hrs. (cm)	Mean (cm)	36 hrs. (cm)	Mean (cm)	42 hrs. (cm)	Mean (cm)	48 hrs. (cm)	Mean (cm)	54 hrs. (cm)	Mean (cm)	60 hrs. (cm)	Mean (cm)	66 hrs. (cm)	Mean (cm)	72 hrs. (cm)	Mean (cm)	78 hrs. (cm)	Mean (cm)	84 hrs. (cm)	Mean (cm)	90 hrs. (cm)	Mean (cm)
<i>vigorous</i>	4.1	4.15	5.9	5.95	9.2	9.25	12.4	12.45	15.4	15.5	17.8	17.9	22.8	22.9	24.5	24.5	27.3	27.4	30.1	30.2	33.4	33.55	35.5	35.6
	4.2		6.0		9.3		12.5		15.6		18.0		23.0		24.7		27.5		30.3		33.7		35.7	
<i>checked</i>	0.7	0.65	1.5	1.45	1.9	1.8	2.7	2.55	3.6	3.45	4.8	4.65	5.3	5.15	5.8	5.6	6.3	6.1	6.7	6.55	7.2	7.05	8.0	7.8
	0.6		1.4		1.7		2.4		3.3		4.5		5.0		5.4		5.9		6.4		6.9		7.7	
<i>plug</i>	5.8	5.7	11.3	11.2	18.2	18.1	23.8	23.65	25.9	25.8	27.9	27.8	29.9	29.8	32.7	32.6	36.0	36.0						
	5.6		11.1		18.0		23.5		29.7		27.7		29.7		32.5		36.0							
<i>ropy</i>	1.6	1.5	2.1	2.0	2.6	2.5	3.5	3.4	4.1	4	4.9	4.8	5.9	5.8	6.2	6.05	6.9	6.75	7.6	7.5	8.3	8.2	8.8	8.8
	1.4		1.9		2.4		3.3		3.9		4.7		5.7		5.9		6.6		7.4		8.1		8.6	
<i>conidial</i>	3.7	3.65	5.8	5.7	8.7	8.6	11.8	11.65	13.5	13.35	15.4	15.25	18.9	18.8	21.0	20.95	23.0	22.9	25.3	25.2	28.4	28.3	30.7	30.6
<i>band</i>	3.6		5.6		8.5		11.5		13.2		15.1		18.7		20.9		22.8		25.1		28.2		30.5	
<i>fluffy</i>	4.6	4.5	5.8	5.7	8.6	8.5	12.3	12.1	15.9	15.7	18.3	18.1	20.2	19.95	24.3	24.05	26.4	26.15	29.0	28.9	33.0	32.9	36.0	36.0
	4.4		5.6		8.4		11.9		15.5		17.9		19.7		23.8		25.9		28.8		32.8		36.0	
<i>crescent</i>	1.5	1.4	2.0	1.9	2.5	2.45	3.4	3.3	4.0	3.9	4.7	4.5	5.8	5.4	6.0	5.9	6.7	6.75	7.4	7.3	8.0	7.9	8.4	8.6
	1.3		1.8		2.4		3.2		3.8		4.3		5.0		5.8		6.8		7.2		7.8		8.8	

Name of the Cultures		Linear growth in cm after the following hours																							
		After 24 hrs. (cm)	Mean (cm)	30 hrs. (cm)	Mean (cm)	36 hrs. (cm)	Mean (cm)	42 hrs. (cm)	Mean (cm)	48 hrs. (cm)	Mean (cm)	54 hrs. (cm)	Mean (cm)	60 hrs. (cm)	Mean (cm)	66 hrs. (cm)	Mean (cm)	72 hrs. (cm)	Mean (cm)	78hrs. (cm)	Mean (cm)	84 hrs. (cm)	Mean (cm)	90hrs. (cm)	Mean (cm)
cauliflower	1.7	1.65	2.2	2.15	2.7	2.65	3.4	3.35	4.0	3.95	4.8	4.75	5.6	5.55	6.4	6.35	7.2	7.15	7.9	7.85	8.6	8.55	9.2	9.1	9.1
	1.6		2.1	2.6		3.3		3.9		4.7		5.5		6.3		7.1		7.8		8.5		9.1			
albino	1.3	1.25	1.6	1.55	1.9	1.85	2.2	2.15	2.5	2.45	3.2	3.15	3.8	3.75	4.4	4.35	5.0	4.95	5.4	5.35	5.8	6.75	6.4	6.3	
	1.2		1.5	1.8		2.1		2.4		3.1		3.7		4.3		4.9		5.3		5.7		6.2			
fluffy band	2.6	2.5	3.2	3.1	4.1	4.0	5.0	4.9	6.1	6.0	7.3	7.2	8.5	8.4	9.7	9.6	11.0	10.9	12.3	12.2	13.6	13.5	15.3	15	
	2.4		3.0	3.9		4.8		5.9		7.1		8.3		9.5		10.8		12.1		13.4		15.1			
Ema	2.3	2.3	4.9	4.9	6.1	6.05	8.3	8.25	11.3	11.25	13.6	13.55	15.9	15.8	17.9	17.8	19.7	19.65	22.2	22.15	24.3	24.25	26.5	26.5	
	2.3		4.9		6.0		8.2		11.2		13.5		15.7		17.7		19.6		22.1		24.2		26.4		

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

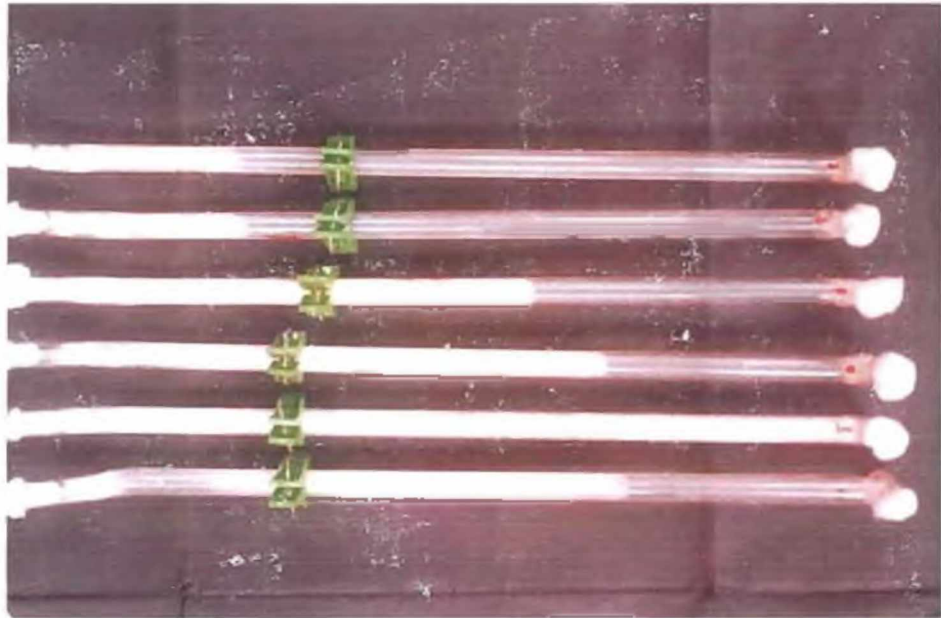


Fig- 43: Linear growth of some selected mutants (*vg, ro, clf, con-band, plug*) with Ema.

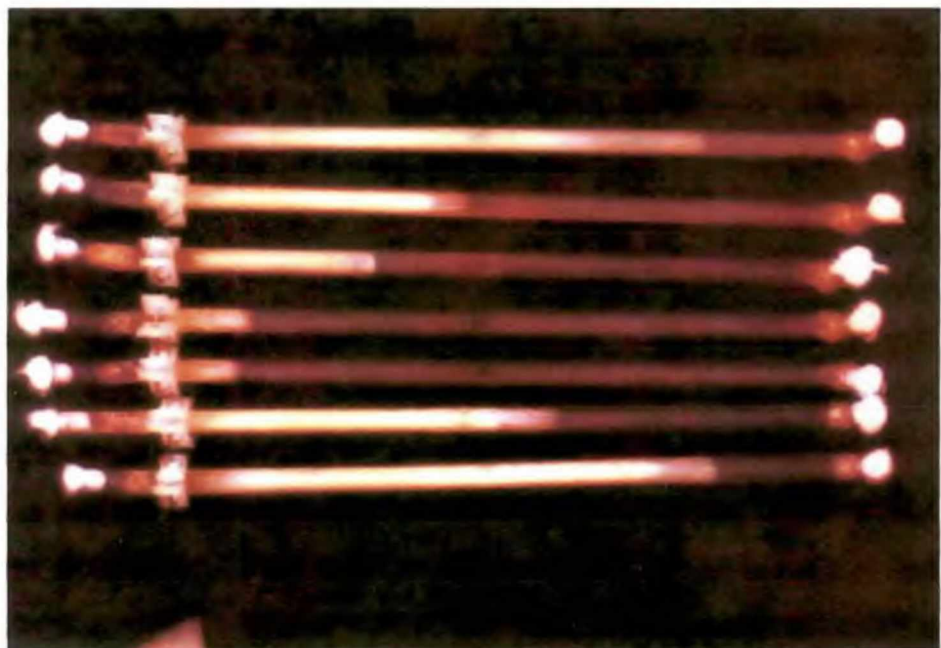


Fig- 44: Linear growth of some selected mutants- *mat, al, crs, fl, ch* with Ema.

Selected mutants were crossed with Ema and EmA separately observation was taken for 4 – 28 days

Table - 20

Mating types and fertility of the selected mutants:

Designation of the cross	Whether perithecia formed Yes/No	Days of initiation of perithecia	frequency of perithecia	Size of perithecia	Mating type	whether spore shed Yes/ No	Shedding days	Fertility
<i>vigorous</i> X Ema	No	- 7	- Many	- Medium	a	- yes	- 19	Excellent
<i>vigorous</i> X Ema A	yes							
<i>vigorous</i> X Ema	No	- 10	- many	- large	a	- yes	- 20	Excellent
<i>vigorous</i> X Ema	Yes							
<i>check</i> X Ema	No	- 10	- Few	- Large	a	- Yes	- 25	Poor
<i>check</i> X Ema	yes							
<i>check</i> X Ema	No	- 6	- Many	- Large	a	- yes	- 15	Excellent
<i>check</i> X Ema	Yes							
<i>ropy</i> X Ema	No	- 6	- few	- Medium	a	- Yes	- 15	Excellent
<i>ropy</i> X Ema	Yes							
<i>ropy</i> X Ema	No	- 13	- Very Few	- large	a	- Yes	- 23	Poor
<i>ropy</i> X Ema	Yes							
<i>conidial</i> X Ema	No	- 6	- Many	- Medium	a	- Yes	- 15	Excellent
<i>bland</i> X Ema	Yes							
<i>con- band</i> X Ema	No	- 12	- very few	- Medium	a	- Yes	- 24	Poor
<i>con- band</i> X Ema	Yes							
<i>fluffyf</i> X Ema	No	- 6	- few	- Medium	a	- yes	- 22	Poor
<i>fluffy</i> X Ema	yes							
<i>fluffy</i> x Ema	No	- 14	- very few	- Medium	a	- yes	- 27	poor
<i>fluffy</i> x Ema	Yes							

Designation of the cross	Whether perithecia formed Yes/No	Days of initiation of perithecia	frequency of perithecia	Size of perithecia	Mating type	whether spore shed Yes/ No	Shedding days	Fertility
<i>crescent</i> X Ema	No	-	-	-	a	-	-	Poor
<i>crescent</i> X Ema	yes	14	Few	Medium		yes	24	
<i>mat</i> X Ema	No	-	-0	-	a	-	-	Poor
<i>mat</i> X Ema	Yes	15	Few	small		Yes	27	
<i>cauliflower</i> X Ema	No	-	-	-	a	-	-	Excellent
<i>cauliflower</i> X Ema	Yes	6	Many	Large		yes	13	
<i>albino</i> X Ema	No	-	-	-	-	-	-	Sterile
<i>albino</i> X Ema	No	-	-	-	-	-	-	
<i>fluffy-band</i> X Ema	No	-	-	-	a	yes	-	Poor
<i>fluffy-band</i> X Ema	yes	13	few	Medium			27	

Table- 21**Segregation ratio of some selected morphological mutants with Em:**

Name of the cross	No. of spores isolated	No of wild types isolated	No of mutant isolated	Segregation ratio. mutant : wild
<i>vigorous</i> X EmA	110	60	50	1t1.2
<i>check</i> X EmA	100	48	52	1.083t1
<i>ropy</i> X EmA	120	62	58	1t.068
<i>con-band</i> X EmA	130	66	64	1t1.031
<i>fluffy</i> X EmA	110	57	53	1t1.075
<i>crescent</i> X EmA	100	47	53	1.127t1
<i>mat</i> X EmA	90	44	46	1.045t1
<i>cauliflower</i> X EmA	100	48	52	1:083t1

Study of Linkage

For the preparation of extraction and for study of linkage, the mutants were crossed with markers of 7 linkage groups. The crosses were made in westergaard's crossing medium with required supplements. The data of the crosses were found as given in the table below.

Table-22

Results of crosses of mutants and markers:-

Name of the mutant	Designation of the cross	Linkage group	Whether perithecia formed Yes/No	Days of initiation of Perithecia	Frequency and Size of the Perithecia	Whether spore shed	Shedding days	Fertility
<i>vigorous</i>	vg×Lue-3A	I	Yes	9	Large, many	Yes	25	Poor
	vg×arg-5A	II	Yes	10	Moderate, Medium	Yes	26	Poor
	vg×trp-1A	III	Yes	8	Many, Medium	Yes	20	Good
	vg×trp-4A	IV	Yes	8	Few, Medium	Yes	27	Poor
	vg×trp-5A	V	Yes	7	Many, Medium	Yes	17	Good
	vg×trp-2A	VI	Yes	10	Few, Small	Yes	19	Good
	vg×arg-10A	VII	Yes	9	Few, Medium	Yes	27	Poor

Name of the mutant	Designation of the cross	Linkage group	Whether perithecia formed Yes/No	Days of initiation of Perithecia	Frequency and Size of the Perithecia	Whether spore shed	Shedding days	Fertility
<i>conidial band</i>	<i>con-band</i> × lue-3A	I	Yes	8	Many, Small	Yes	19	Good
	<i>con-band</i> × arg-5A	II	Yes	9	Few, Medium	Yes	26	Poor
	<i>con-band</i> × trp-1A	III	Yes	8	Many, Small	Yes	20	Good
	<i>con-band</i> × trp-4A	IV	Yes	10	Few, Large	Yes	25	Poor
	<i>con-band</i> × trp-5A	V	Yes	8	Some, large	Yes	18	Good
	<i>con-band</i> × trp-2A	VI	Yes	9	Few, Medium	Yes	20	Good
	<i>con-band</i> × arg-10A	VII	Yes	8	Some, Small	Yes	21	Good
<i>fluffy</i>	<i>fluffy</i> × lue-3A	I	Yes	8	Many, Small	Yes	17	Good
	<i>fluffy</i> × arg-5A	II	Yes	7	Few, Medium	Yes	18	Good
	<i>fluffy</i> × trp-1A	III	Yes	9	Some, medium	Yes	20	Good
	<i>fluffy</i> × trp-4A	IV	Yes	10	Some, Small	Yes	21	Good
	<i>fluffy</i> × trp-5A	V	Yes	8	Many, Large	Yes	19	Good
	<i>fluffy</i> × trp-2A	VI	Yes	10	Many, Small	Yes	21	Good
	<i>fluffy</i> × arg-10A	VII	Yes	9	Few, Medium	Yes	16	Good

Name of the mutant	Designation of the cross	Linkage group	Whether perithecia formed Yes/No	Days of initiation of Perithecia	Frequency and Size of the Perithecia	Whether spore shed	Shedding days	Fertility
<i>ropy</i>	<i>ropy</i> × <i>leu-3A</i>	I	Yes	8	Some, Medium	Yes	16	Good
	<i>ropy</i> × <i>arg-5A</i>	II	Yes	9	Many, Small	Yes	18	Good
	<i>ropy</i> × <i>trp-1A</i>	III	Yes	8	Many, Small	Yes	16	Good
	<i>ropy</i> × <i>trp-4A</i>	IV	Yes	12	Many, Medium	Yes	26	Poor
	<i>ropy</i> × <i>trp-5A</i>	V	Yes	10	Very Few, Small	Yes	27	Poor
	<i>ropy</i> × <i>trp-2A</i>	VI	Yes	13	Few, Medium	Yes	25	Poor
	<i>ropy</i> × <i>arg-10A</i>	VII	Yes	11	Few, Medium	Yes	20	Good
<i>mat</i>	<i>mat</i> × <i>leu-3A</i>	I	Yes	9	Few, Medium	Yes	20	Good
	<i>mat</i> × <i>arg-5A</i>	II	Yes	8	Many, Large	Yes	19	Good
	<i>mat</i> × <i>trp-1A</i>	III	Yes	8	Many, Large	Yes	17	Good
	<i>mat</i> × <i>trp-4A</i>	IV	Yes	8	Some, Large	Yes	21	Good
	<i>mat</i> × <i>trp-5A</i>	V	Yes	8	Many, Small	Yes	19	Good
	<i>mat</i> × <i>trp-2A</i>	VI	Yes	9	Few, Medium	Yes	18	Good
	<i>mat</i> × <i>arg-10A</i>	VII	Yes	9	Few, Large	Yes	19	Good

Name of the mutant	Designation of the cross	Linkage group	Whether perithecia formed Yes/No	Days of initiation of Perithecia	Frequency and Size of the Perithecia	Whether spore shed	Shedding days	Fertility
<i>cauliflower</i>	<i>clf.</i> × <i>leu-3A</i>	I	Yes	12	Many, Medium	Yes	26	Poor
	<i>clf.</i> × <i>arg-5A</i>	II	Yes	8	Some, Medium	Yes	17	Good
	<i>clf.</i> × <i>trp-1A</i>	III	Yes	7	Few, Medium	Yes	17	Good
	<i>clf.</i> × <i>trp-4A</i>	IV	Yes	10	Some, Small	Yes	25	Poor
	<i>clf.</i> × <i>trp-5A</i>	V	Yes	8	Many, Medium	Yes	21	Good
	<i>clf.</i> × <i>trp-2A</i>	VI	Yes	11	Few, Medium	Yes	27	Poor
	<i>clf.</i> × <i>arg-10A</i>	VII	Yes	7	Few, Large	Yes	16	Good
<i>checked</i>	<i>check</i> × <i>luc-3A</i>	I	Yes	13	Few, Medium	Yes	26	Poor
	<i>check</i> × <i>arg-5A</i>	II	Yes	8	Some, Medium	Yes	27	Poor
	<i>check</i> × <i>trp-1A</i>	III	Yes	10	Very Few, Medium	Yes	22	Good
	<i>check</i> × <i>trp-4A</i>	IV	Yes	12	Few, Medium	Yes	26	Poor
	<i>check</i> × <i>trp-5A</i>	V	Yes	9	Few, Medium	Yes	21	Good
	<i>check</i> × <i>trp-2A</i>	VI	Yes	8	Many, Medium	Yes	20	Good
	<i>check</i> × <i>arg-10A</i>	VII	Yes	7	Many, Medium	Yes	21	Good

Name of the mutant	Designation of the cross	Linkage group	Whether perithecia formed Yes/No	Days of initiation of Perithecia	Frequency and Size of the Perithecia	Whether spore shed	Shedding days	Fertility
<i>crescent</i>	<i>crescent</i> × <i>leu-3A</i>	I	Yes	8	Some, Small	Yes	17	Good
	<i>crescent</i> × <i>arg-5A</i>	II	Yes	8	Few, Small	Yes	19	Good
	<i>crescent</i> × <i>trp-1A</i>	III	Yes	9	Few, Small	Yes	21	Good
	<i>crescent</i> × <i>trp-4A</i>	IV	Yes	10	Many, Medium	Yes	27	Poor
	<i>crescent</i> × <i>trp-5A</i>	V	Yes	10	Few, Small	Yes	26	Poor
	<i>crescent</i> × <i>trp-2A</i>	VI	Yes	9	Few, Medium	Yes	27	Poor
	<i>crescent</i> × <i>arg-10A</i>	VII	Yes	9	Large, Many	Yes	21	Good
<i>fluffy band</i>	<i>fluffy band</i> × <i>leu-3A</i>	I	Yes	8	Large, Many	Yes	19	Good
	<i>fluffy band</i> × <i>arg-5A</i>	II	Yes	7	Medium, Same	Yes	15	Good
	<i>fluffy band</i> × <i>trp-1A</i>	III	Yes	9	Medium, Same	Yes	22	Good
	<i>fluffy band</i> × <i>trp-4A</i>	IV	Yes	10	Medium, Same	Yes	19	Good
	<i>fluffy band</i> × <i>trp-5A</i>	V	Yes	8	Medium, Same	Yes	17	Good
	<i>fluffy band</i> × <i>trp-2A</i>	VI	Yes	8	Medium, Same	Yes	21	Good
	<i>fluffy band</i> × <i>arg-10A</i>	VII	Yes	9	Large, Many	Yes	20	Good

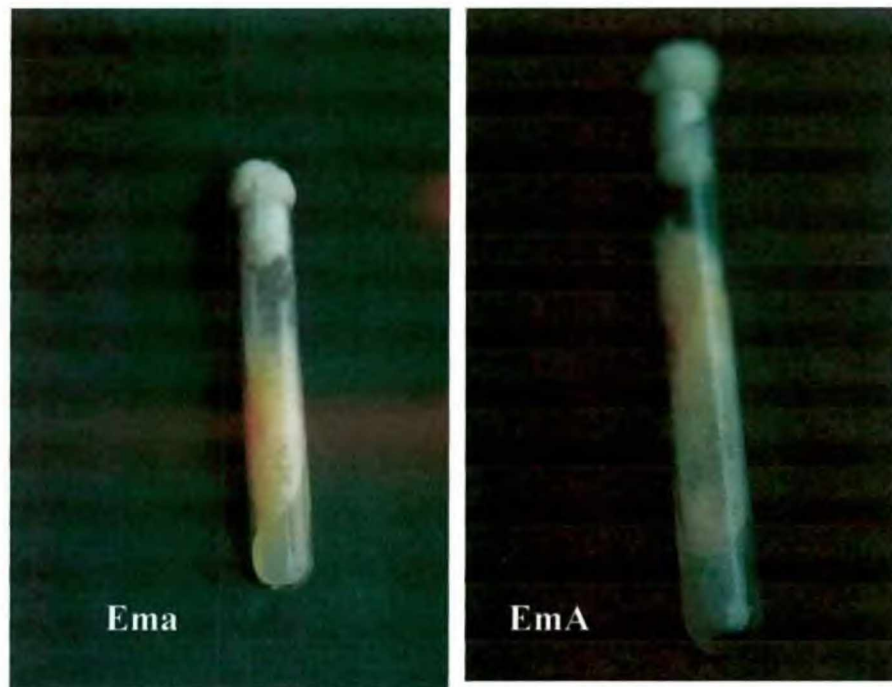


Fig :- 45. Ema (5297) and EmA (5296)



Fig :- 46. A fertile cross.

Determination of linkage of selected mutants:

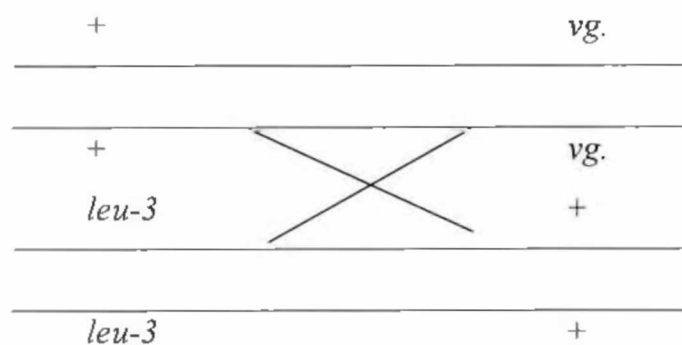
Table-23

Linkage of *vigorous*:

Marker used	Linkage group	Crossing name	Total isolates	Progenies	% of the progenies	Inference on linkage
<i>leu-3A</i>	I	<i>vg. × leu-3A</i>	94	a. <i>leu-3A</i> =32 b. <i>vg.</i> = 36 c. Wild = 18 d. <i>vg.+leu-3A</i> =8	a. 34.04% b. 38.30% c. 19.15% d. 8.51%	Linked with <i>leu-3A</i> of linkage group-I.
<i>trp-1A</i>	III	<i>vg. × trp-1A</i>	70	a. <i>trp-1A</i> =16 b. <i>vg.</i> = 18 c. Wild = 15 d. <i>vg.+trp-1A</i> =21	a. 22.86% b. 25.71% c. 21.43% d. 30%	Not linked with linkage group-III
<i>trp-4A</i>	IV	<i>vg. × trp-4A</i>	98	a. <i>trp-4A</i> =21 b. <i>vg.</i> = 24 c. Wild = 25 d. <i>vg.+trp-4A</i> =28	a. 21.43% b. 24.49% c. 25.51% d. 28.57%	Not linked with linkage group-IV
<i>trp-2A</i>	VI	<i>vg. × trp-2A</i>	96	a. <i>trp-2A</i> =20 b. <i>vg.</i> = 22 c. Wild = 28 d. <i>vg.+trp-2A</i> =26	a. 20.83% b. 22.92% c. 29.17% d. 27.08%	Not linked with linkage group-IV
<i>arg-10A</i>	VII	<i>vg. × arg-10A</i>	95	a. <i>arg-10A</i> =22 b. <i>vg.</i> = 23 c. Wild = 24 d. <i>vg.+arg-10A</i> =26	a. 23.16% b. 24.21% c. 25.26% d. 27.37%	Not linked with linkage group-VII

: Determination of Genetic Map of *vigorous* mutant.

Crossing name: *vigorous* (K₄470a) × Leu-3 (R156) A



$$\begin{aligned}
 \text{Distance} &= \frac{R_1 + R_2}{\text{Total progenies}} \times 100(\text{centimorgan}) \\
 &= \frac{18 + 8}{94} \times 100(\text{centimorgan}) \\
 &= 26.51 \text{ CM.}
 \end{aligned}$$

Here,

$R_1 + R_2 = \text{Recombinants}$

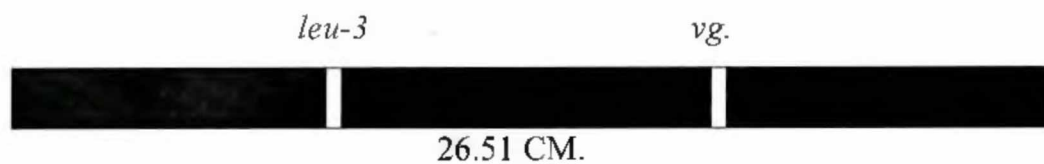


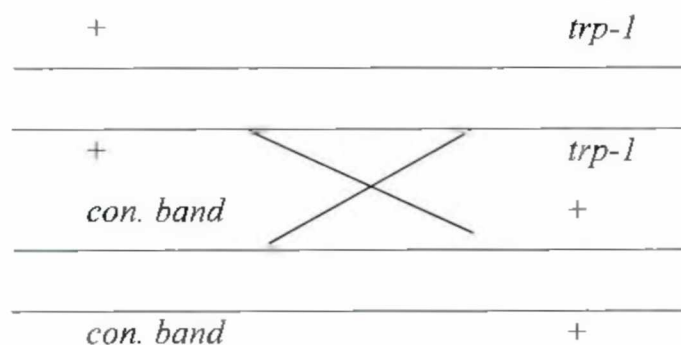
Fig-47: Genetic Map of *vigorous* (K₄470a) mutant.

Table-24**Linkage of conidial band:**

Marker used	Linkage group	Crossing name	Total isolates	Progenies	% of the progenies	Inference on linkage
<i>leu-3A</i>	I	<i>con-band</i> × <i>leu-3A</i>	89	a. Wild = 25 b. <i>leu-3A</i> = 21 c. <i>con-band</i> = 15 d. <i>con-band</i> + <i>leu-3A</i> = 28	a. 28.09% b. 23.6% c. 16.85% d. 31.46%	Not linked with linkage group-I
<i>trp-1A</i>	III	<i>con-band</i> × <i>trp-1A</i>	72	a. Wild = 11 b. <i>trp-1A</i> = 23 c. <i>con-band</i> = 25 d. <i>con-band</i> + <i>trp-1A</i> = 13	a. 15.28% b. 31.94% c. 34.72% d. 18.06%	Linked with <i>trp-1A</i> of linkage group-III
<i>trp-4A</i>	IV	<i>con-band</i> × <i>trp-4A</i>	75	a. Wild = 14 b. <i>trp-4A</i> = 9 c. <i>con-band</i> = 23 d. <i>con-band</i> + <i>trp-4A</i> = 29	a. 18.67% b. 12% c. 30.67% d. 38.67%	Not linked with linkage group-IV
<i>trp-2A</i>	VI	<i>con-band</i> × <i>trp-2A</i>	87	a. Wild = 20 b. <i>trp-2A</i> = 22 c. <i>con-band</i> = 19 d. <i>con-band</i> + <i>trp-2A</i> = 26	a. 22.99% b. 25.29% c. 21.84% d. 29.89%	Not linked with linkage group-VI
<i>arg-10A</i>	VII	<i>con-band</i> × <i>arg-10A</i>	51	a. Wild = 13 b. <i>arg-10A</i> = 12 c. <i>con-band</i> = 10 d. <i>con-band</i> + <i>arg-10A</i> = 16	a. 25.49% b. 23.53% c. 19.6% d. 31.37%	Not linked with linkage group-VII

: Determination of Genetic Map of *conidial band* mutant.

Crossing name: *conidial band* (K₂299a) × *trp-1* (106) A



$$\begin{aligned}
 \text{Distance} &= \frac{R_1 + R_2}{\text{Total progenies}} \times 100(\text{centimorgan}) \\
 &= \frac{11+13}{72} \times 100(\text{centimorgan}) \\
 &= 29.05 \text{ CM.}
 \end{aligned}$$

Here,

$R_1 + R_2 = \text{Recombinants}$

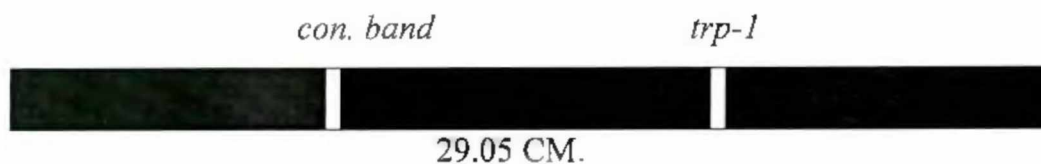


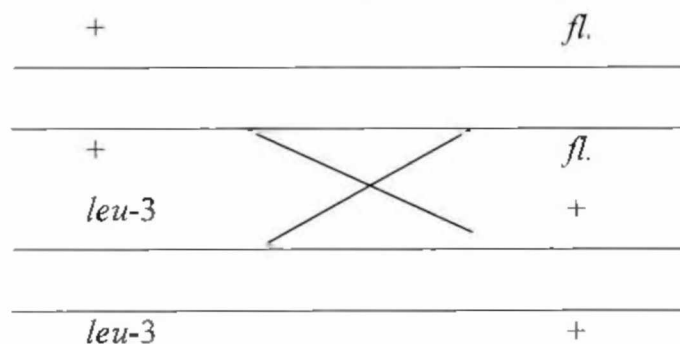
Fig:-48. Genetic Map of *conidial band* (K₂299a) mutant.

Table-25Linkage of *fluffy*:

Marker used	Linkage group	Crossing name	Total isolates	Progenies	% of the progenies	Inference on linkage
<i>leu-3A</i>	I	<i>fluffy</i> × <i>leu-3A</i>	57	a. Wild = 11 b. <i>leu-3A</i> = 20 c. <i>fluffy</i> = 23 d. <i>fluffy</i> + <i>leu-3A</i> = 3	a. 19.3% b. 35.09% c. 40.35% d. 5.26%	Linked with <i>leu-3A</i> of linkage group-I
<i>trp-1A</i>	III	<i>fluffy</i> × <i>trp-1A</i>	90	a. Wild = 30 b. <i>trp-1A</i> = 20 c. <i>fluffy</i> = 22 d. <i>fluffy</i> + <i>trp-1A</i> = 18	a. 33.31% b. 22.22% c. 24.44% d. 20%	Not linked with linkage group-III
<i>trp-4A</i>	IV	<i>fluffy</i> × <i>trp-4A</i>	71	a. Wild = 22 b. <i>trp-4A</i> = 10 c. <i>fluffy</i> = 21 d. <i>fluffy</i> + <i>trp-4A</i> = 18	a. 30.99% b. 14.08% c. 29.58% d. 25.35%	Not linked with linkage group-IV
<i>trp-2A</i>	VI	<i>fluffy</i> × <i>trp-2A</i>	84	a. Wild = 24 b. <i>trp-2A</i> = 17 c. <i>fluffy</i> = 17 d. <i>fluffy</i> + <i>trp-2A</i> = 26	a. 28.57% b. 20.24% c. 20.24% d. 30.95%	Not linked with linkage group-VI
<i>arg-10A</i>	VII	<i>fluffy</i> × <i>arg-10A</i>	71	a. Wild = 23 b. <i>arg-10A</i> = 18 c. <i>fluffy</i> = 21 d. <i>fluffy</i> + <i>arg-10A</i> = 09	a. 32.39% b. 25.35% c. 29.58% d. 12.67%	Not linked with linkage group-VII

: Determination of Genetic Map of *fluffy* mutant.

Crossing name: *fluffy* (C₃366a) × *leu-3* (R156)A



$$\begin{aligned}
 \text{Distance} &= \frac{R_1 + R_2}{\text{Total progenies}} \times 100(\text{centimorgan}) \\
 &= \frac{11+3}{57} \times 100(\text{centimorgan}) \\
 &= 16.26 \text{ CM.}
 \end{aligned}$$

Here,

$R_1 + R_2 = \text{Recombinants}$

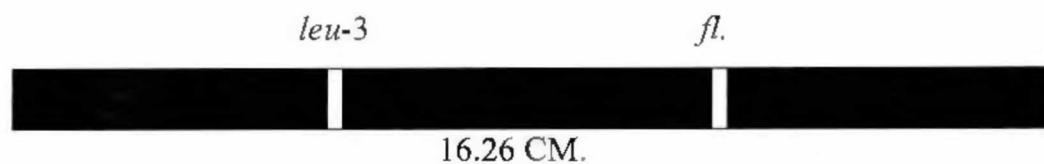


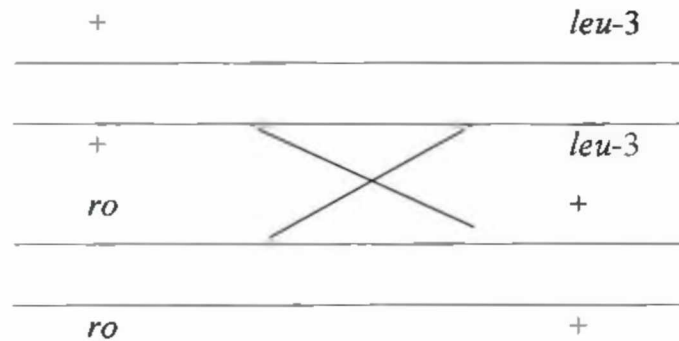
Fig:- 49. Genetic Map of *fluffy* (C₃366a) mutant.

Table-26**Linkage of *ropy*:**

Marker used	Linkage group	Crossing name	Total isolates	Progenies	% of the progenies	Inference on linkage
<i>leu-3A</i>	I	<i>ro</i> × <i>leu-3A</i>	85	a. Wild = 14 b. <i>leu-3A</i> =28 c. <i>ro</i> =27 d. <i>ro</i> + <i>leu-3A</i> =16	a. 16.47% b. 32.94% c. 31.76% d. 18.82%	Linked with <i>leu-3A</i> of linkage group-I
<i>trp-1A</i>	III	<i>ro</i> × <i>trp-1A</i>	82	a. Wild = 23 b. <i>trp-1A</i> =35 c. <i>ro</i> = 8 d. <i>ro</i> + <i>trp-1A</i> =16	a. 28.05% b. 42.68% c. 9.76% d. 19.51%	Not linked with linkage group-III
<i>trp-4A</i>	IV	<i>ro</i> × <i>trp-4A</i>	84	a. Wild = 13 b. <i>trp-4A</i> =23 c. <i>ro</i> =11 d. <i>ro</i> + <i>trp-4A</i> =37	a. 15.48% b. 27.38% c. 13.09% d. 44.05%	Not linked with linkage group-IV
<i>trp-2A</i>	VI	<i>ro</i> × <i>trp-2A</i>	86	a. Wild = 21 b. <i>trp-2A</i> =22 c. <i>ro</i> =19 d. <i>ro</i> + <i>trp-2A</i> =24	a. 24.42% b. 25.58% c. 22.09% d. 27.9%	Not linked with linkage group-VI
<i>arg-10A</i>	VII	<i>ro</i> × <i>arg-10A</i>	66	a. Wild = 20 b. <i>arg-10A</i> =11 c. <i>ro</i> =24 d. <i>ro</i> + <i>arg-10A</i> =11	a. 30.3% b. 16.66% c. 36.36% d. 16.66%	Not linked with linkage group-VII

: Determination of Genetic Map of *ropy* mutant.

Crossing name: *ropy* (K₄409a) × *leu-3* (R156)A



$$\begin{aligned}
 \text{Distance} &= \frac{R_1 + R_2}{\text{Total progenies}} \times 100(\text{centimorgan}) \\
 &= \frac{14 + 16}{85} \times 100(\text{centimorgan}) \\
 &= 32.82 \text{ CM.}
 \end{aligned}$$

Here,

$R_1 + R_2 = \text{Recombinants}$

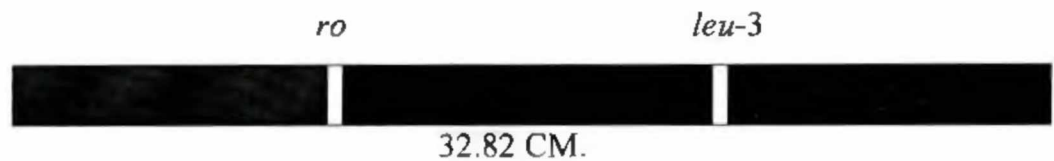


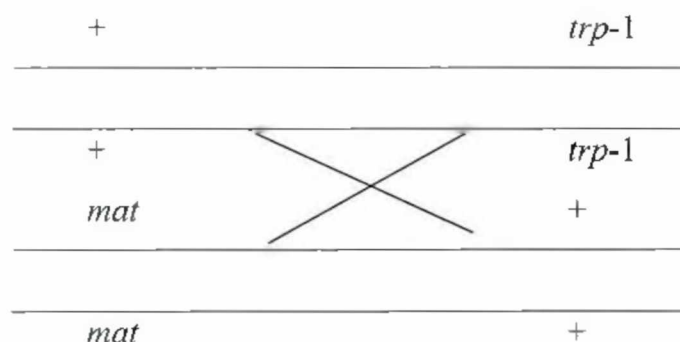
Fig:-50. Genetic Map of *ropy* (K₄409a) mutant.

Table-27Linkage of *mat*:

Marker used	Linkage group	Crossing name	Total isolates	Progenies	% of the progenies	Inference on linkage
<i>leu-3A</i>	I	<i>mat</i> × <i>leu-3A</i>	92	a. Wild = 27 b. <i>leu-3A</i> =26 c. <i>mat</i> =18 d. <i>mat</i> + <i>leu-3A</i> =21	a. 29.34% b. 28.26% c. 19.56% d. 22.82%	Not linked with <i>leu-3A</i> of linkage group-I
<i>trp-1A</i>	III	<i>mat</i> × <i>trp-1A</i>	92	a. Wild = 9 b. <i>trp-1A</i> =35 c. <i>mat</i> = 37 d. <i>mat</i> + <i>trp-1A</i> =11	a. 9.78% b. 38.04% c. 40.21% d. 11.95%	Linked with <i>trp-1A</i> of linkage group-III
<i>trp-4A</i>	IV	<i>mat</i> × <i>trp-4A</i>	77	a. Wild = 5 b. <i>trp-4A</i> =23 c. <i>mat</i> =22 d. <i>mat</i> + <i>trp-4A</i> =27	a. 6.49% b. 29.87% c. 28.57% d. 35.06%	Not linked with <i>trp-4A</i> of linkage group--IV
<i>trp-2A</i>	VI	<i>mat</i> × <i>trp-2A</i>	72	a. Wild = 18 b. <i>trp-2A</i> =23 c. <i>mat</i> =26 d. <i>mat</i> + <i>trp-2A</i> =25	a. 25% b. 31.94% c. 36.11% d. 34.72%	Not linked with <i>trp-2A</i> of linkage group-VI
<i>arg-10A</i>	VII	<i>mat</i> × <i>arg-10A</i>	88	a. Wild = 22 b. <i>arg-10A</i> =17 c. <i>mat</i> =29 d. <i>mat</i> + <i>arg-10A</i> = 20	a. 25% b. 19.31% c. 32.95% d. 22.72%	Not linked with <i>arg-10A</i> of linkage group-VII

: Determination of Genetic Map of *mat* mutant.

Crossing name: *mat* (S₄411a) × *trp*-1 (106) A



$$\begin{aligned}
 \text{Distance} &= \frac{R_1 + R_2}{\text{Total progenies}} \times 100(\text{centimorgan}) \\
 &= \frac{9 + 11}{92} \times 100(\text{centimorgan}) \\
 &= 20.956 \text{ CM.}
 \end{aligned}$$

Here,

$R_1 + R_2 = \text{Recombinants}$

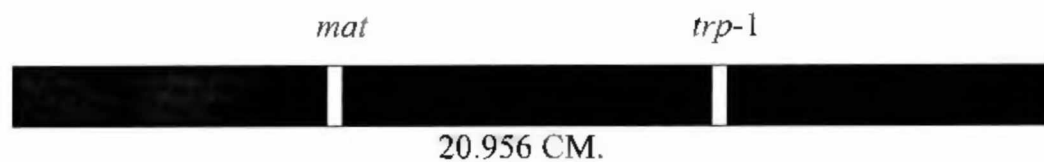


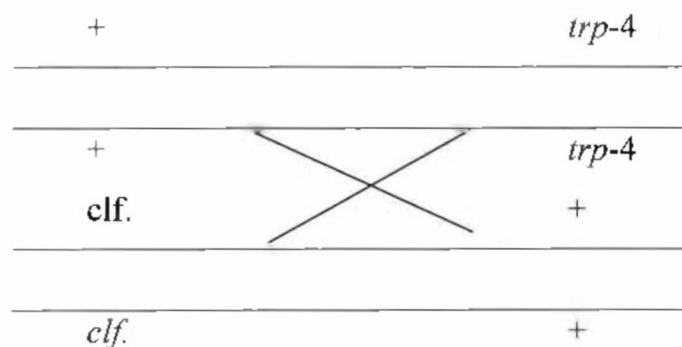
Fig:-51. Genetic Map of *mat* (S₄411a) mutant.

Table-28Linkage of *cauliflower*:

Marker used	Linkage group	Crossing name	Total isolates	Progenies	% of the progenies	Inference on linkage
<i>leu-3A</i>	I	<i>clf.</i> × <i>leu-3A</i>	62	a. Wild = 16 b. <i>leu-3A</i> =12 c. <i>clf.</i> =16 d. <i>clf.</i> + <i>leu-3A</i> =18	a. 25.8% b. 19.35% c. 25.8% d. 29.03%	Not linked with <i>leu-3A</i> of linkage group-I
<i>trp-1A</i>	III	<i>clf.</i> × <i>trp-1A</i>	92	a. Wild = 26 b. <i>trp-1A</i> =22 c. <i>clf.</i> = 21 d. <i>clf.</i> + <i>trp-1A</i> =23	a. 28.26% b. 23.91% c. 22.82% d. 25%	Not Linked with <i>trp-1A</i> of linkage group-III
<i>trp-4A</i>	IV	<i>clf.</i> × <i>trp-4A</i>	58	a. Wild = 5 b. <i>trp-4A</i> =21 c. <i>clf.</i> =23 d. <i>clf.</i> + <i>trp-4A</i> =9	a. 8.62% b. 36.2% c. 39.65% d. 15.51%	Linked with <i>trp-4A</i> of linkage group--IV
<i>trp-2A</i>	VI	<i>clf.</i> × <i>trp-2A</i>	64	a. Wild = 15 b. <i>trp-2A</i> =18 c. <i>clf.</i> =12 d. <i>clf.</i> + <i>trp-2A</i> =19	a. 23.43% b. 28.12% c. 18.75% d. 29.68%	Not linked with <i>trp-2A</i> of linkage group-VI
<i>arg-10A</i>	VII	<i>clf.</i> × <i>arg-10A</i>	75	a. Wild = 21 b. <i>arg-10A</i> =20 c. <i>clf.</i> =15 d. <i>clf.</i> + <i>arg-10A</i> = 19	a. 28% b. 26.66% c. 20% d. 25.33%	Not linked with <i>arg-10A</i> of linkage group-VII

: Determination of Genetic Map of *cauliflower* mutant.

Crossing name: *cauliflower* (U256) × *trp-4* (Y2198) A



$$\begin{aligned}
 \text{Distance} &= \frac{R_1 + R_2}{\text{Total progenies}} \times 100(\text{centimorgan}) \\
 &= \frac{5 + 9}{58} \times 100(\text{centimorgan}) \\
 &= 20.517 \text{ CM.}
 \end{aligned}$$

Here,

$R_1 + R_2 = \text{Recombinants}$

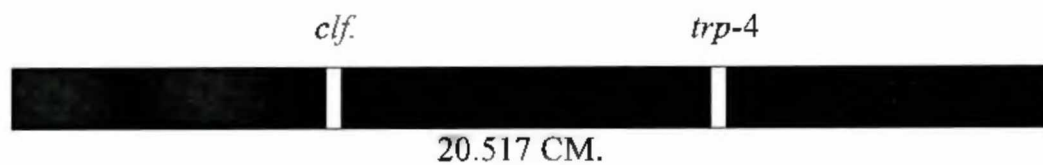


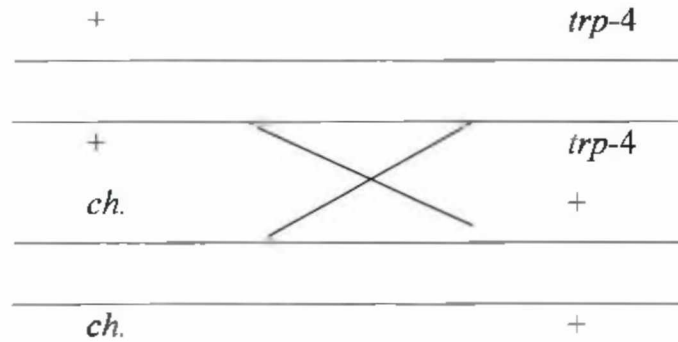
Fig:-52. Genetic Map of *cauliflower* (U256) mutant.

Table-29**Linkage of checked:**

Marker used	Linkage group	Crossing name	Total isolates	Progenies	% of the progenies	Inference on linkage
<i>leu-3A</i>	I	<i>ch.</i> × <i>leu-3A</i>	61	a. Wild = 27 b. <i>leu-3A</i> =10 c. <i>ch.</i> =15 d. <i>ch.</i> + <i>leu-3A</i> =9	a. 44.26% b. 16.39% c. 24.59% d. 14.75%	Not linked with <i>leu-3A</i> of linkage group-I
<i>trp-1A</i>	III	<i>ch.</i> × <i>trp-1A</i>	70	a. Wild = 21 b. <i>trp-1A</i> =21 c. <i>ch.</i> = 12 d. <i>ch.</i> + <i>trp-1A</i> =16	a. 30% b. 30% c. 17.14% d. 22.85%	Not Linked with <i>trp-1A</i> of linkage group-III
<i>trp-4A</i>	IV	<i>ch.</i> × <i>trp-4A</i>	90	a. Wild = 9 b. <i>trp-4A</i> =33 c. <i>ch.</i> =31 d. <i>ch.</i> + <i>trp-4A</i> =17	a. 10% b. 36.66% c. 34.44% d. 18.88%	Linked with <i>trp-4A</i> of linkage group--IV
<i>trp-2A</i>	VI	<i>ch.</i> × <i>trp-2A</i>	73	a. Wild = 25 b. <i>trp-2A</i> =14 c. <i>ch.</i> =23 d. <i>ch.</i> + <i>trp-2A</i> =11	a. 34.24% b. 19.17% c. 31.5% d. 15.06%	Not linked with <i>trp-2A</i> of linkage group-VI
<i>arg-10A</i>	VII	<i>ch.</i> × <i>arg-10A</i>	57	a. Wild = 29 b. <i>arg-10A</i> =15 c. <i>ch.</i> =10 d. <i>ch.</i> + <i>arg-10A</i> = 3	a. 50.87% b. 26.31% c. 17.54% d. 5.26%	Not linked with <i>arg-10A</i> of linkage group-VII

: Determination of Genetic Map of *checked* mutant.

Crossing name: *checked* (T₃317a) × *trp-4* (Y2198) A



$$\begin{aligned}
 \text{Distance} &= \frac{R_1 + R_2}{\text{Total progenies}} \times 100(\text{centimorgan}) \\
 &= \frac{9 + 17}{90} \times 100(\text{centimorgan}) \\
 &= 27.88 \text{ CM.}
 \end{aligned}$$

Here,
R₁+R₂ = Recombinants

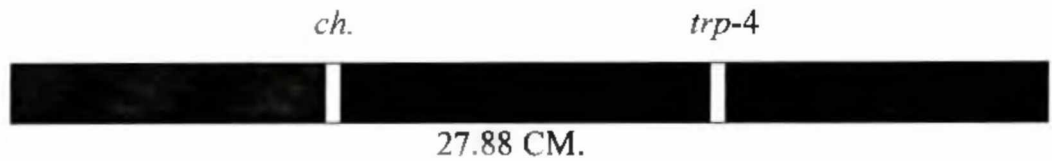


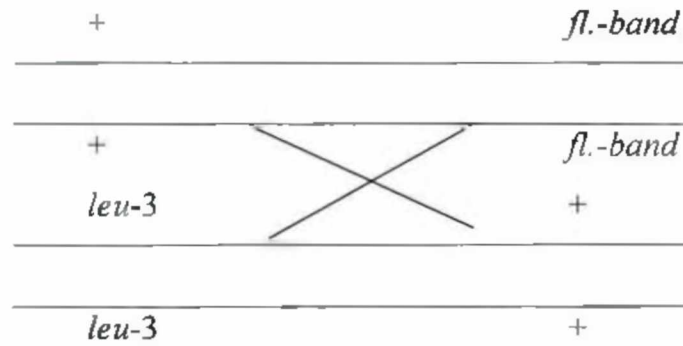
Fig:-53. Genetic Map of *checked* (T₃317a) mutant.

Table-30Linkage of *fluffy band*:

Marker used	Linkage group	Crossing name	Total isolates	Progenies	% of the progenies	Inference on linkage
<i>leu-3A</i>	I	<i>fl.-band</i> × <i>leu-3A</i>	61	a. Wild = 13 b. <i>leu-3A</i> =21 c. <i>fl.-band</i> =23 d. <i>fl.-band</i> + <i>leu-3A</i> =4	a. 21.31% b. 34.42% c. 37.7% d. 6.55%	Not linked with <i>leu-3A</i> of linkage group-I
<i>trp-1A</i>	III	<i>fl.-band</i> × <i>trp-1A</i>	76	a. Wild = 26 b. <i>trp-1A</i> =16 c. <i>fl.-band</i> = 20 d. <i>fl.-band</i> + <i>trp-1A</i> =14	a. 34.21% b. 21.05% c. 26.31% d. 18.42%	Linked with <i>trp-3A</i> of linkage group--I
<i>trp-4A</i>	IV	<i>fl.-band</i> × <i>trp-4A</i>	71	a. Wild = 23 b. <i>trp-4A</i> =08 c. <i>fl.-band</i> =22 d. <i>fl.-band</i> + <i>trp-4A</i> =18	a. 32.39% b. 11.26% c. 30.98% d. 25.35%	Not Linked with <i>trp-1A</i> of linkage group-III
<i>trp-2A</i>	VI	<i>fl.-band</i> × <i>trp-2A</i>	77	a. Wild = 22 b. <i>trp-2A</i> =16 c. <i>fl.-band</i> =15 d. <i>fl.-band</i> + <i>trp-2A</i> =24	a. 28.57% b. 20.77% c. 19.48% d. 31.16%	Not linked with <i>trp-2A</i> of linkage group-VI
<i>arg-10A</i>	VII	<i>fl.-band</i> × <i>arg-10A</i>	63	a. Wild = 21 b. <i>arg-10A</i> =16 c. <i>fl.-band</i> =19 d. <i>fl.-band</i> + <i>arg-10A</i> = 7	a. 33.33% b. 25.39% c. 30.15% d. 11.11%	Not linked with <i>arg-10A</i> of linkage group-VII

: Determination of Genetic Map of *fluffy band* mutant.

Crossing name: *fluffy band* (C₄452a) × *leu-3* (R156) A



$$\begin{aligned}
 \text{Distance} &= \frac{R_1 + R_2}{\text{Total progenies}} \times 100(\text{centimorgan}) \\
 &= \frac{13 + 4}{61} \times 100(\text{centimorgan}) \\
 &= 19.557 \text{ CM.}
 \end{aligned}$$

Here,
R₁+R₂ = Recombinants

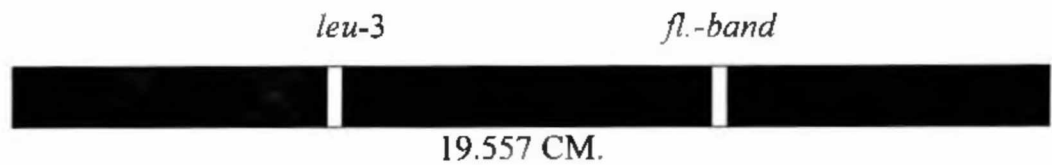
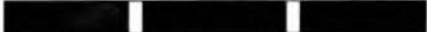


Fig:-54. Genetic Map of *fluffy band* (C₄452a) mutant.

Table-31**Distance of the mutant with the marker:**

Mutant used	Marker used	Linkage group of the marker	Distance of the mutant with marker
vg. k ₄ 470a	leu-3 (R156)A	I	leu-3 vg.  26.51 CM.
con-band k ₂ 299a	trp-I (106A)	III	Con. band trp-I.  29.05 CM.
fl. C ₃ 366a	leu-3 (R156)A	I	leu-3 fl.  16.26 CM.
ro. k ₄ 409a	leu-3 (R156)A	I	ro leu-3  32.82 CM.
mat. S ₄ 411a	trp-I (106A)	III	mat trp-I  20.956 CM
clf. U256	trp-4 (Y2198)A	IV	clf. trp-4  20.517 CM.
ch. T ₃ 317a	trp-4 (Y2198)A	IV	ch. trp-4  27.88 CM.
fl. band C ₄ 452a	leu-3 (R156)A	I	leu-3 fl. band  19.557 CM

DISCUSSION

DISCUSSION

Spontaneous mutation in *Neurospora crassa* is very rare and infrequent in nature (David *et al* 1993; Stadler *et. al.* 1993; Waters and Stadler, 1995) . The author checked if there was any spontaneous mutation in *Neurospora crassa*. In this investigation conidia from fresh culture of Ema (5297) were spread. Colonies were isolated individually in VM tubes. After 4-5 days when sufficient conidia were formed, individual cultures were analyzed. None of the isolates were found to show morphological variations and all of them resembled the parental stock.

It seems that there was no spontaneous mutation in *Neurospora crassa* in laboratory stock which the author used for the induction of mutation. The previous research workers of microbial genetics Laboratory, Department of Botany, University of Dhaka also did not obtain any spontaneous mutations (Hossain 1995). Induction of mutation in *Neurospora crassa* with aqueous extract of garlic (*Allium sativum* Linn.) and genetical and chromatographic studies of some selected mutants (Mozmader *et al* 2000).

To test the effectiveness of *Ocimum sanctum* Linn. (Tulshi) on the radial growth of *Neurospora crassa* T_p, T₁, T₂, T₃, T₄ and T₀ concentrations were used. It was evident from Table-5 that the radial growth of *Neurospora crassa* was proportional to the concentrations of Tulshi used. By increasing the concentration of leaf extract of *Ocimum sanctum* Linn. radial growth of Ema was reduced in comparison to control.

T_p concentration was found to be more effective than T₁, T₂, T₃, T₄ and T₀ concentrations. An extensive study was made of the amount of different concentrations that *Neurospora crassa* will tolerate with little checked growth and also of the amount necessary to kill the fungus *Neurospora crassa*.

Then some mutants induced with leaf extract of Tulshi were selected for further study.

Radial growth of the wild type and some selected Tulshi induced mutants were studied after 6 hours upto 48 hours, after 48 hours wild type Ema (5297) grew upto 3.6 cm where as mutant plug grew upto 4.4 cm and albino grew upto 0.9 cm only.

An experiment on the mutagenicity of Tulshi was performed with the T_p concentration for induction of mutation. 9 groups of mutants were obtained (i.e. *ro*, *al*, *vg*., *con-band*, *fl*, *ch*, *mat*, *clf*, *dir*.), mutants *ropy* showed highest frequency (15.789%) and lowest frequency (6.578%) was given by the mutants *dirty*.

To test the effectiveness of *Averrhoa carambola* Linn. (Kamranga) on the radial growth of *Neurospora crassa* different concentrations of kamranga fruit extracts were used (k_p , k_1 , k_2 , k_3 , k_4 , k_5 , k_6). It was evident that the radial growth of *N. crassa* was proportional to the concentrations of kamranga used. By increasing the concentration of fruit extracts of *Averrhoa carambola* Linn. radial growth of Ema was reduced in comparison to control. K_p concentration was found more effective than k_1 , k_2 , k_3 , k_4 and k_6 concentrations. conidia of Ema (5297) of *N. crassa* were treated for 6 hours separately in 100% concentration of the extracts. 12 groups of mutants (i.e. *ro*, *al*, *pl*, *vg*, *con-band*, *fl*, *fl-band*, *ch*, *mat*, *y-fl*, *clf*, *org*.) were obtained with fruit extract of *Averrhoa carambola* Linn., mutants *ropy* showed highest frequency (14.14%) and lowest frequency (2.02%) was given by the mutants *orange*.

The author also used leaf extrat of shetadron for induction of mutation. To test the effectiveness of *Leucas lavandulaefolia* Rees. (Shetadron) on the radial growth of *N. crassa* different concentrations of shetadron leaf extracts were used (S_p , S_1 , S_2 , S_3 , S_4 and S_0). It was evident that the radial growth of *N. crassa* was proportional to the concentrations of shetadron used. By increasing the concentration of leaf extracts of *Leucas lavandulaefolia* Rees. (shetadron) radial growth of Ema was reduced in comparison to control. S_p concentration was found more effective than S_1 , S_2 , S_3 , S_4 and S_0

concentrations. Conidia of Ema (5297) of *N. crassa* were treated for 6 hours separately in 100% concentration of the extracts. 11 groups of mutants (i.e. - *ro*, *pl*, *vg*, *al*, *con*, *band*, *fl*, *ch*, *clf*, *fl-band* and *dir*) were obtained with leaf extract of *Leucas lavandulaefolia* Rees., mutants *con-band* and *ropy* showed highest frequency (12.79%) and lowest frequency (2.325%) was given by the mutant *dirty*. To test the effectiveness of *Coccinea cordifolia* Linn. (Telakucha) on the radial growth of *N. crassa*, different concentrations of Telakucha leaf extracts were used (c_p , c_1 , c_2 , c_3 , c_4 and c_0). It was evident that the radial growth of *N. crassa*, was proportional to the concentrations of Telakucha used. By increasing the concentration of leaf extracts of *Coccinea cordifolia* Linn. radial growth of Ema was reduced in comparison to control. c_p concentration was found more effective than c_1 , c_2 , c_3 , c_4 and c_0 concentrations. Conidia of Ema (5297) of *N. crassa* were treated for 6 hours separately in 100% concentration of the extracts. 10 groups of mutants (i.e. - *al*, *vg*, *ro*, *pl*, *fl*, *mat*, *con-band*, *clf*, *chand fl-band*) were obtained with leaf extracts of (*Coccinea cordifolia* Linn.) mutants *al*, *vg* and *con-band* showed highest frequency (12.195%) and lowest frequency (3.658%) was given by the mutants *cauliflower*.

During the present work the author also used UV light (254nm) for artificial induction of mutation. Irradiation of conidia at a distance of 15cm for 2, 2½ and 3 minutes under UV light found to be effective. 12 groups of mutants were obtained which were *ro*, *al*, *mat pl* *vg*, *fl*, *con-band*, *crescent*, *orange*, *clf*, *fl-band* and *dir*. *ropy* showed the highest frequency (12.676%) and the lowest frequency (1.408%) showed by *orange* and *dirty*. This result is slightly different from the work Suktana (1997). A good number of morphological mutants induced with leaf extract of *Ocimum sanctum* L., *Leucas lavandulaefolia* Rees., *Coccinea cordifolia* Linn. and fruit extract of *Averrhoa carambola* Linn. and UV irradiation were selected for detailed study and analysis. The mutants showed variation with Ema in their

morphology, weight of the mycelia, linear growth rate, initiation of conidial germination, fertility, mating type, segregating ratio etc. were also checked.

Radial growth of the wild type and some selected induced mutants were studied after 6 hours upto 48 hours, after 48 hours wild type Ema (5297) grew upto 3.6 cm where as mutant *plug* grew upto 4.4cm and *albino* grew upto 0.9cm only.

Growth on race tubes (Ryan. et al 1943) is also useful for accurate measurement of growth and growth rate. Linear growth of some selected mutants were taken after 6 hours upto 84 hours. After 72 hours the linear growth of Ema was 19.6cm where as the maximum growth 36cm was obtained in case of *albino*.

Time required for conidial germination of selected mutants were recorded upto 6 hours. It was shown that there was no indication of germination of conidia or mycelial growth upto $2\frac{1}{2}$ hours, *plug* germinated checked and *ropy* germinated after $5\frac{1}{2}$ hours. Mating types and fertility of selected induced mutants were determined. The mutant did not show variations in their mating types but different mutant behaved differently, majority of them were excellent and some of them were poor in their fertility. But only a few of them were completely sterile. The selected mutants were crossed with Em'a' and Em'A' separately and their segregating ratio was studied. Mating type of some mutant was also checked. The progenies showed about 1:1 ratio in their morphology and mating type. So it was concluded that the mutant characters have resulted due to mutation in *nuclear gene*.

Complementation of different and similar morphological mutants were done to see whether the mutants were allelic or not. The results were negative when similar morphological mutant were used but positive when different morphological mutants were used.

Linkage study of *vg* (k₄-470), *con-band* (k₂-299), *fl*(c₃-366), *ro* (k₄-409), *mat* (s₄-411), *clf* (u-256), *ch*(T₃-317) and *fl-band* (c₄-452) were undertaken by crossing them with seven makers representative of all the seven linkage groups of *leu-3*(R156)A, *arg-5*(27947)A, *trp-I*(106)A, *trp-4*(Y2198)A, *trp-5*(A420)A, *trp-2*(75001)A and *arg-10*(B317)A.

vg, *fl* and *ropy* are linked with *leu-3* of linkage group-I; *con-band* and *mat* are linked with *trp-I* of linkage group-III; *clf* and *ch* are linked with *trp-4* of linkage group-IV.

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