REMOVAL OF ENVIRONMENTAL POLLUTANTS USING *NEUROSPORA CRASSA* BIOMASS

A DISSERTATION SUBMITTED FOR THE DEGREE OF
MASTER OF PHILOSOPHY
TO THE DEPARTMENT OF BOTANY

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October, 2010.

DEDICATED TO

My Father
Md. Mahbubar Rahman
and
My Mother
Mrs. Mazeda Mahbub

CERTIFICATE

This is to certify that this thesis contains the results of research on "**Removal of Environmental Pollutants Using *Neurospora crassa* biomass**" and has been carried out by Mahbuba Akhter Jahan under our supervision. It is further certified that the work presented here is original and suitable for submission as an M.Phil thesis.

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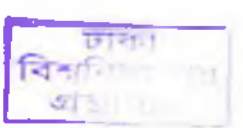

DECLARATION

I hereby declare that this thesis entitled "**Removal of Environmental Pollutants Using *Neurospora crassa* Biomass**" has been composed by myself and all the works presented herein are my own. I further declare that this work has not been submitted anywhere for any academic degree.

Mahbuba Akhter Jahan
28.10.10

October, 2010 **Mahbuba Akhter Jahan**

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ACKNOWLEDGEMENT

In the name of Allah.This work, on "Removal of environmental pollutants using *Neurospora crassa* biomass" has been carried out in the Microbial Genetics Laboratory of the Department of Botany, University of Dhaka under the guidance of my supervisors Dr. Tahsina Rahim and Dr. T.I.M.A. Mozmader, Professor, Department of Botany, University of Dhaka.

I must express my debt of gratitude to my respectable supervisors for giving me the problem and also for their constant guidance, advice and encouragement without which my work would never have been completed. I am grateful to my supervisor Dr. Tahsina Rahim for her kind help.She observed my slightest difficulties with utmost keenness and extended her sympathetic hand to override all difficulties in all possible ways. I am also thankful to my supervisor Dr. T.I.M.A. Mozmader because of his valuable constructive suggestion and continuing guidence.

I wish to acknowledge with many thanks to my respectable teacher Dr. Abul Hassan, chairman, Dr.M.R. Khan Professor of Botany, University of Dhaka for providing all research facilities. I want to acknowledge with gratefulness to my respectable teacher Abdul Hafiz Mollah, Ex-Head of the department of Botany, Carmichael College, Rangpur, who helped to buildup my basic platform in Botany.

My thanks are also due to Mazeda Begum, Director and Nasima Khan, Principal Scientific officer of BCSIR for their help to test single pollutants (As & Pb). I am thankful to S M Faisal, Lecturer CIT, IUT and Masuda Akhter,Lecturer Law and Human Rights,UAP for their cordial help.

I am thankful to Dewan Md. Atiqur Rahman Milon, VP Prime Bank, Gazipur for his support and co-operation. My thanks are for Md. Akhlak Hossain for his cordial support and encouragement. I am also grateful to Apurba Lal Ray for his significant co-operation.

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LIST OF ABBREVIATIONS

al	albino
clf	cauliflower
cb	conidial band
cm	centimeter
Em	Emerson stock
Ema	Emerson'a'
EmA	Emerson'A'
fi	fissure
fl	fluffy
fl band	fluffy band
g	gram
mg	miligram
ml	mililitre
ro	ropy
SM	Sorbose Minimal .
UV	Ultra Violet
Vg	Vigorous
VM	Vogel's Minimal .
%	Percentage

ABSTRACT

Environmental pollutants which are the great environmental issue for human health and other living organism as well as the environment itself, are to be removed by using the component of environment. *Neurosporoa crassa*, a filamentous non-pathogenic fungus possesses interesting characteristics which is called BIOSORPTION. The biosorption capacity of *N. crassa* and its mutants were used to remove the possible environmental pollutants.

Three plants were used to get mutants of *N. crassa*. These were leaf extract of papaya (*Carica papaya* Linn), fruit extract of bitter gourd (*Momordica charantia* Linn) and leaf extract of tea (*Camelia sinensis* Linn. O. Kuntze). Mutation of *N. crassa* with leaf extract of *Carica papaya* resulted 5 types of mutants namely *vigorous, cauliflower, fluffy, fluffy-band* and *conidial- band*. Fruit extract of *Momordica charantia* with *N. crassa* resulted 4 types of mutants namely *albino dirty, ropy* and *vigorous*. Mutation of *N. crassa* with leaf extract of *Camelia sinensis* resulted 4 types of mutant's namely *conidial-band, fissure, fluffy* and *plug*.

Beside chemical mutagen, UV ray, as a physical mutagen also used for mutation of *N. crassa*. By UV irradiation, 4 types of mutants were obtained namely *vigorous, fluffy, conidial-band* and *cauliflower*.

The pollutants were divided as Single and Mixed. Single pollutants are arsenic (As) and lead (Pb). Mixed pollutants are Dye pollutants and Tannery pollutants.

Out of 10 mutants *vigorous* showed highest growth and it was 2.5 times higher than the wild type. Mutant *vigorous* was used to test Single and Mixed pollutants. Wild type *N. crassa* (Ema) was also used for the same purpose.

Result was analysed qualitatively and quantitatively. In case of Single pollutants, biomass of the mutant *vigorous* showed no biosorption capacity. In case of Mixed pollutants, biomass of the organism showed significant biosorption capacity. This implied that the organism is suitable for removal of those environmental pollutants.

INTRODUCTION

Introduction

Now a day's environmental pollution is the burning question of the world. Any abnormal change in chemical, physical and biological characteristics of environment is called pollution. At present our civilization is getting polluted every moment. This is causing extensive, damage to all sorts of life on earth. This damaging effect is now so extensive and increasing at such a rapid pace that pollution is now the most widely discussed topic of the world today.

Human numbers, wealth, poverty, technology and beliefs are having planet-wide consequences. The energy and agricultural scenarios have several large environmental implication, thus the world community is concerned about the future of human society itself.

Environment of particular place refers to an aggregate of the geosphere and biosphere obtaining in that place. So, the state of environment of that place is the function of the quantitative and qualitative state of a member of variable indicators of the geosphere and biosphere pertaining to that place.

The variable indicators in geosphere include land, air, and water systems with non-living and non-biological resources in it, whereas those in biosphere are human beings, various other biological systems as various microorganisms, wildlife, croplands, forests, grasslands, wetlands and fisheries etc. Hence any examination of the state of environment requires taking into account the state of these indicators constituting the overall environmental system.

Environmental pollution is caused primarily by human that introduce extraneous substances into the environment thereby causing unfavorable changes. This changes in turn effect human by endangering his health, harming the ecosystem and by

interfering with legitimate use of the environmental resources, the immediate effects of environmental pollution could be seen as- health problems by affecting human health and lives, economic problems by affecting the value of human property and materials, ecological problems by disturbing eco-balance, interfering with conservation of natural resources and threatening the existence of precise; and aesthetic problem by affecting human sense.

In these days of environmentally conscious world community, it is the duty of the biologist to find the utilization of many non-conventional naturally occruing fast growing species for the benefit of mankind. For this regard, *N. crassa* has proved to be an important material for its profuse vegetative growth in synthetic media. The most conspicuous feature of the fungus is the great abundance of mitotically produced pink spores as conidia, (Burnett-1975)

N. crassa is a filamentous fungus that belongs to the class Ascomycetes, sub-class-pyrenomycitade, order-xylariales, Family-sordariaceae. The vegetative phase consists of a mycelium of branching hyphae and only the zygote is diploid. *N. crassa* is octosporous, heterothallic and as a result there are two mating type called 'A' and 'a'.

It has distinct male and female sexual structure. It produces great masses of brilliant, orange-red asexual spores. It is non pathogenic and can be easily grown on a suitable mixture of mineral salts in water plus a carbon source (Sucrose) and the vitamin biotin (Beadle and Tatum, 1941; Annonymous, 1966). Both growth on racetube (Ryan *et al.* 1943) and dry weight of mycelia (Beadle and Tatum, 1941) are useful for accurate measurement of growth. The former measuring rate and the latter

terminal growth mass. It has short life cycle. It can complete its vegetative life cycle within 3-4 days and sexual cycle within 15 days.

The phenomenon of 'biosorption' has been well established and it is being practiced in different applied fields (Gadd and Griffiths 1987, Hutchins *et al.* 1986, Wilson *et al.* 1992) Research with fungus like *Neurospora* always yields a considerable amount of biomass which is disposed of as waste. But the biosorption experiment can be conducted to find out the possibilities of using theses so called wastes to remove hazardous chemical pollutants form our environment. Lately workers have pointed out that pollutants like arsenic, chromium, lead, mercury nickel, and other pollutant are posing threats to the public health in Bangladesh.

While workers in the different fields are proposing to use physical, chemical and biological methods (Edward- 1993, Meng and Latterman -1993, Soda *et al.* 2009, Edwards *et al* -2008. Escudero *et al* .2009, Rao *et al* 2009, Wang *et al* .2009, Beak *et al*.2009,Kim *et al.* 2009, Borah *et al*.2009, Qu, *et al*.2009, Gupta *et al* 2009, De *et al*.2009, Cundy *et al* .2008, Annibaldi *et al* 2009, Sahu *et al* .2009, Bongiorni *et al.* 2008, Goh *et al.* 2009, Tapas *et al.* 1999, Giles *et al.* 1974,Sastry and Madhavakrishna.1984, kaul and Nandy. 1991, Gupta *et al.* 2002, Gupta *et al* .2005, Ruthven -1984,Mondal *et al*.2008)for the detoxification of our environment particularly the soil and natural water bodies. The microbs can also be exploited for the same purpose. Living microbs continue interacting with their environment throughout their life span but even after their death the biomass can successfully be used to adsorb and remove pollutants from their liquid phase and thus clearing our environment.

It was proposed to carry out experiments with *N. crassa* biomass to evalute its potentiality for the removal of Environmental pollutants. The author has divided the pollutants into two parts: single pollutants which are determine or mixed in the laboratory with appropriate proportion (eg. As. Pb) and mixed pollutants which contains more than one chemicals and other substances (eg, Dye pollutants and Tannery pollutants).

Mozmader *et al* (1997) induced with formaldehyde solution a fast growing mutant that gave a growth of 1.4-2.2 times more biomass than that of wild type (Ema). Mozmader *et al* .(2002) also obtained much higher dry weight of mycelia in shaken culture and in media containing water from dry coconut. But that mutant was not utilized for the study of the capacity of removing environmental pollutants.

The author has induced mutation with physical and chemical mutagen to get mutant. 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 Garcia and Padilla (1996), Haque and Samsi(a) 1996,Haque and Samsi(b)1996, Gupta *et al.* 1976, Bashar and Rai- 1991, Natarjan and Lalithakumari- 1987, Singh and Pandey- 1989, Tiwari *et al.* 1987, Ray and Rahim-2010, Ray *et al.* 2010, Miah *et al*- 1990, Mishra and Dixit- 1979, Lokendra and Sharma- 1978, Ahmed and Sultana- 1984, Alice and Rao- 1986, Rajeshawri and Mariappan -1992, Shiekh and Ognihotri -1972, Sahana -1997, Nanir and Kadu. 1987, allover the worlds have been studying the plant extract for such purpose.

Ultra Violet light seems to induce true gene mutations as well as losses reversion to the wild type gene and perhaps also mutation to other alleles. The maximum absorption of UV by DNA is at a wave length of 254 nm. Maximum mutagenecity also occurs at 254 nm. UV radiation of 254 nm was used for induction of mutation.

In research on microorganisms UV radiation surves the purpose more effectively (Catcheside- 1954, Hollaender *et al.* 1945, Hossain- 1935). Most of the mutants for the study of gene actions in microorganism were produced by exposure of cells to UV light (Auerbach.1962). In the Genetic research laboratory of Dhaka University, Ahmad and Mozmader in 1977 irradiated the conidia of *N. crassa* strain Emerson a (5297) with UV light and isolated a numbers of biochemical mutants which are studied by Begum (1966).

The author has induced mutation with plant extract and UV ray. 10 mutants were obtained form those mutations. Out of those *vigorous* mutant showed highest growth. Wild type (Ema) and vigorous mutant were selected for the test of bisorption capacity. In the case of As and Pb *vigorous* showed insignificant biosorption capacity. On the other hand Ema and vigorous showed significant capacity to Tannery and Dye pollutant.

MATERIALS

MATERIALS

The experimental material was *Neurospora crassa*, an Ascomycetes fungus. It exists as a Soil Saprophyte. *N. crassa* can grow on sucrose bases, carbon vegetation or in bakeries. It is 'Pink bread Mold (Shear and Dodge, 1927), this fungus possesses interwind segmented filaments called hyphae. Each hyphal segment contains a haploid nucleus with seven chromosomes. It reproduces asexually by haploid spores called conidia and sexually by the contact of hyphae of two opposite mating types or strains, known as EmA and Ema.

Culture Emerson a (Ema 5297), Emerson A (EmA), 5296) were used for this investigation. Ema was used for treating conidia with mutagen and EmA and Ema were used for checking the mating types of the mutants and extracts.

The wild type strains of *N. crassa* were kindly supplied by Prof. T.I. M.A. Mozmader from Genetic Stock, Dept. of Botany, University of Dhaka.

1.1 Wild Types

a) Emerson A (EmA, 5296)

b) Emerson a (Ema 5297)

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

Apparatus

1. Autoclave
2. Centrifuge
3. Electric-balance
4. Fluorescence Microscope
5. Incubator
6. Inoculation chamber
7. Refrigerator
8. UltraViolet lamp.
9. Shaker.

Glass Wares

Name	Made	Size	Purpose
1. Test tubes	Pyrex	0.5"× 2 "	Isolating tubes
2. Test tubes	Pyrex	0.5"×3"	Sub culturing tubes
3. Test tubes	Pyrex	5/8" × 5"	Stock tubes
4. Test tubes	Pyrex	1.5 ×36cm	growth (race tube)
5. Test tubes	Pyrex	8"×1"	Radiation tube
6. Petridishes	Pyrex	1.5" dia	Irradiation
7. Petridishes	Pyrex	3" dia	Media
8. Flasks (Erlenmyer)	Pyrex	100 ml	Media and Water etc.
9. Flasks (Erlenmyer)	Pyrex	250 ml	Media and water etc.
10. Flasks (Erlenmyer)	Pyrex	500 ml	Media and water etc.
11. Flasks (Enlermyer)	Pyrex	100 ml	Media and water etc.

Chemical used in the research work

General

1. Agar (Bacto)
2. Ammonium nitrate [NH_4NO_3]
3. Biotin [$C_{10}H_{16}N_2O_3S$]

4. Boric Acid [H_3PO_4] anhydride
5. Calcium Chloride [$CaCl_2$, $6H_2O$]
6. Chloroform [$CHCL_3$]
7. Citric Acid [$C(OH)(COOH)CCH_2COOH)_2$, H_2O]
8. Copper Sulphate [$CuSO_4$, $7H_2O$]
9. Ferrous Sulphate [$FeSO_4$, $7H_2O$]
10. Glucose ($C_6H_{12}O_6$)
11. Magnesium Sulphate [$MgSO_4$, $7H_2O$]
12. Manganese Sulphate [$MnSO_4$, $7H_2O$]
13. Methanol [Ch_3OH]
14. Potassium Di-Hydrogen Phosphate [KH_2PO_4]
15. Potassium Nitrate [KNO_3]
16. Sodium Chloride [NaCl]
17. Sodium Citrate [$NaH_2C_6H_5O_7$, $2H_2O$]
18. Sodium Molybdate [Na_2MoO_4, $2H_2O$]
19. Sorbose [$OCH_2(CHOH)_3$]
20. Sucrose [$C_{12}H_{22}O_{11}$]
21. Zinc Sulphate [$ZnSO_4$, $7H_2O$]

Supplements

1. Adenine
2. Arginine
3. Histidine
4. Leucine
5. Lysine
6. Tryptophan

Mutagen

A. Physical Mutagen:

a) UV Irradiation of 254 nm.

B. Chemical Mutagen:

a) Papaya leaf extract (*Carica papaya* Linn)
Family-Caricaceae.

b) Bitter gourd fruit extract (*Momordica charantia* Linn)
Family- Cucurbitaceae.

c) Tea leaf extracts (*Camelia sinensis* Linn. O. Kuntze)
Family-Theaceae

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 (Beadle 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 was kept in refrigerator. All media were sterilized in the autoclave at 120^0C under 15 lb pressure for 20 minutes.

Composition of Trace elements solution per 100 ml:

1. Distilled water	95 ml
2. Citric acid C(OH)(COOH) ($CH_2COOH)H_2O$	5 gm
3. Zinc Sulphate ($ZnSO_4$, $7H_2O$)	5 gm
4. Ferrous Sulphate ($FeSO_4$,$6H_2O$)	1.0 gm
5. Copper Sulphate ($CuSO_4$,$5H_2O$)	0.25 gm
6. Manganese Sulphate ($MnSO_4$, H_2O)	0.65 gm
7. Boric acid anhydrous (H_3BO_4)	0.05 gm
8. Sodium molybdate ($NaMoO_4$,$2H_2O$)	0.05 gm
9. Chloroform ($Chcl_3$)	1 ml

This solution needed for Vogel's Stock Solution.

Composition of Biotin Solution per 100 ml

1. Distilled water	100ml
2. Biotin	10 mg

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

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 litre.

1. Distilled water	770 ml
2. Sodium Citrate 2.$H_2$0	63.5 gm
3. Potassium dihydrogen phosphate (KH_2PO_4)	125 g
4. Ammonium Nitrate (NH_4NO_3)	50g
5. Magnesium Sulphate ($MgSO_4$ $7H_2O$)	5g
6. Calcium Chloride ($CaCl_2.6H_2$0)	3.75 g
7. Trace Elements Solution	2.5 ml
8. Biotin Solution	1.25 ml
9. Chloroform	2 ml

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

Stock solution of supplements

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. ryptophan 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 100 ml conical flask. For frequent use, solution was kept in test tubes fitted with sterilized pipettes. The solution in the pipettes was used drop wise.

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, 10 ml 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 eg leucine medium, adenine medium etc.

Composition of Vogel's Minimal Medium (VM) per litre for culturing

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

This medium was used for subculturing 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. 100 mg of required amino acid was used for supplemented VM per litre.

Vogel's Minimal Liquid Medium

1. Distilled water	960 ml
2. Vogel's Stock Solution	40 ml
3. Sucrose	20 g.

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

Medium for determining linear growth

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

The media was supplemented where necessary.

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 colony and single spore needed to be isolated. The problem was circumvented by the finding that when certain chemicals, notably the Ketohexose Sorbose had been added to the culture media, a restricted colonial growth habit results (Tatum *et al.* 1949). The importance of the ratio between other carbon sources and sorbose had been investigated (Tatum *et al.* 1949, Brockman and De Serres. 1964, Pittenger. 1964) for that reason supplemented Sorbose minimal medium as well as non supplemented Sorbose minimal medium was used for plating. This medium was also used for minimal test of wild type and mutant.

Composition of Sorbose minimal medium (SM) per litre

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

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

METHODS

METHOD

Stock Maintaining

Usually 6 inches tubes containing 4 ml of required medium were used for maintaining the stock of wild type, and mutant. After incubation period the inoculated tubes were preserved in the refrigerator at $\pm$ 4^{0}C.

Subculturing

Subculturing was important to obtain fresh cultures and for preservation. Generally 2½"–3" tubes were used for this purpose. 2 ml sterilized Vogel's Minimal Medium or Vogel's Supplemented Medium (Where supplement was necessary) was used for subculturing. Wild type (Ema and EmA) and morphological mutant were subcultured in Vogel's Minimal medium. Bio-chemical mutants were subcultured in supplemented VM. Particular amino acid supplements were used for particular biochemical mutants.

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^{0}C for 4-5 days for the growth and formation of conidia.

SPONTANEOUS MUTATION IN Neurospora crassa

Spontaneous mutation in *Neurospora crassa* is very infrequent in nature (Stadler *et al.*1993, David *et al.*1993, Watters *et al.*1995). It was observed if there was any spontaneous mutation in *Neurospora crassa*. There were several steps for finding out the spontaneous mutation in *N. crassa*.

(1) Obtaining of fresh culture of Ema

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

(2) 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.

(3) 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 10 ml sterilized distilled water was added and was shaken well.

4) 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 47^0C was added in each petridish. The conidial suspension and SM media were mixed by shaking gently. Then plates were incubated at 25^0C for growth of conidia.

5) Isolation

After 17 hours single colonies were isolated in small tubes containing VM medium. 2000 copies were isolated and incubated at 25^0C for proper growth.

6. Observation

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

Study of Mugagenic Effect of Leaf Extract of Papaya (*Carica papaya* Linn) on *Neurospora crassa*

Preparation of solution of different concentration of papaya (*Carica papaya* Linn).

Experiment was made to find out the mutagenic effect of papaya leaf extract on *Neurospora crassa*. Papaya extracts of different density were prepared. 120 gm leave of papaya were taken washed 3-4 times with tap water and 3 times with distilled water. After air drying the leaf were cutted into small pieces and grinded in an electric grinder. The paste was filtreted with a sterilized cotton cloth and finally filtred with filter paper to get clear solution.

All extract namely 0.5, 1.0, 2.0, 4.0 were prepared separately in sterilized testube in refrigerator.

For testing the effect of papaya extract on the growth of *Neurospora crassa* with different concentration of papaya solution were prepared. Different solutions were taken separately on the petridish at the rate of 0.5 ml, 1.0 ml, 2.0 ml, 4.0 ml and only 10 ml of VM on a petridish. 10 ml of molten VM was added in each petridish and rotated gently for uniform mixing of solution with medium. When the medium became solid the centre of the petridish was marked and fresh culture of Ema was inoculated at that point with a sterilized needle.

All the petridishes were kept in incubator at 25^{0}C. After 18, 24, 30 and 36 hours the radial growth of Ema of each petridish was measured in cm

Treating of Ema with extract of papaya leaf.

a) Obtaining of fresh culture

To obtain fresh culture, Ema was cultured 4 times after 4 days intervals in each case. 5 days old culture was used for treating conidia.

b) Sterilization

All the media, essential elements and instruments were sterilized in the autoclave at 120^{0}c under 15 lb pressures for 30 minutes. The inoculation chamber, needle, centrifuge machine etc. were sterilized with rectified spirit.

c) Centrifugation

10 ml leaf extract of the concentration was taken into 2 centrifuge tubes. 1 loop of conidia of Ema was taken into each tube and was shaken for homogenous solution. The solution was centrifuged with the help of a centrifuge machine for 15-20 minutes.

d) Filtration

After centrifugation the solution above conidia was poured out from the centrifuge tube. 10 ml of sterilized distilled water was added in the centrifuge tube and centrifuged for 3 minutes. Then the distill water was pouered out. The same procedure was repeated 2 times.

e) Preparation of suspension with treated conidia

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

f) Plating of treating conidia

The sterilized Petridishes were marked and 1 drop of the suspension was taken accordingly. 10 ml of molten SM media was added to a petridish and were shaken gently to mix the suspension and media. The plates were kept inside the incubator at 25^0c for growth of conidia.

g) Isolation of Single Conidial Colony

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

h) Classification

After 5 days all the cultures were observed and classified by comparing their characters with wild type Ema.

The conidial cultures which had any morphological variations were subcultured several times in small tubes and checked carefully whether any permanent morphological change has taken place. The mutants were selected for further analysis, growth of mycelia, colour of hyphae and conidia etc. were considered while selection was made

i) Growth test of different morphological mutants on SM, VM

Growth tests were made in Sterilized petridishes containing Sorbose minimal medium, Vogel's minimal medium. 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.

Study of Mutagenic Effect of Fruit Extract of bitter gourd (*Momordica charantia* Linn) on *Neurospora crassa.*

Preparation of Solution of different concentration of Bitter gourd (*Momordica charantia* Linn)

Experiment was made to find out the mutagenic effect of Bitter gourd fruit extract on *Neurospora crassa* bitter gourd extract of different density were prepared. 60 gm fruits of bitter gourd were taken. Washed 3 times with tap water and 3 times with distilled water. After air drying the fruits were cutted into small pieces and grinded in an electric grinder. The paste was filtrated with a sterilized cotton cloth and finally filtred with filter paper to get clean solution.

All extract namely 0.5, 1.0, 2.0, 4.0 were prepared separately in sterilized test tube in refrigerator.

Effect of extract of Bitter gourd on the growth of *Neurospora crassa* .

For testing the effect of Bitter gourd extract on the growth of *Neurospora crassa* with different concentration of bitter solution were prepared. Different solutions were taken separately on the petridish at the rate of 0.5 ml, 1.0 ml, 2.0 ml, 4.0 ml and only 10 ml of VM on a petridish. 10 ml of molten VM was added in each peridish and rotated gently for uniform mixing of solution with medium. When the medium became solid the centre of the petridish was marked and fresh culture of Ema was inoculated at that point with a sterilized needle.

All the petridishes were kept in incubator at 25^0C. After, 18, 24, 30 and 36 hours the radial growth of Ema of each petridish was measured in cm.

Treating of Ema with extract of bitter gourd fruits

a) Obtaining of fresh culture

To obtain fresh culture Ema was cultured 4 times after 4 days intervals in each case. 5 days old culture was used for treating conidia.

b) Sterilization

All the media, essential elements and instruments were steirlized in the autoclave at 120^0c under 15 lb pressures for 30 minutes. The inoculation chamber, needle, centrifuge machine etc. were sterilized with rectified spirit.

c) Centrifugation

10 ml fruit extract of the concentration were taken into 2 centrifuge tubes. 1 loop of conidia of Ema was taken into each tube and was shaken for homogenous solution. The solutions were centrifuged with the help of a centrifuge machine for 15-20 minutes.

d) Filtration

After centrifugation the solution above conidia was poured out from the centrifuge tube. 10 ml of sterilized distilled water was added in the centrifuge tube and centrifuge for 3 minutes. Then the distilled water was poured out. The same procedure was repeated 2 times.

e) Preparation of suspension with treated conidia

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

f) Plating of treating conidia

The sterilized petricdishes were marked and 1 drop of the suspension was taken accordingly. 10 ml of molten SM media was added to a petridish and were shaken gently to mix the suspension and media. The plates were kept inside the incubator at 25^{0}c for growth of conidia.

g) Isolation of single condial colony

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

h) Classification

After 5 days all the cultures were observed and classified by comparing their characters with wild types Ema.

The conidial cultures which had any morphological variations were subcultured several times in small tubes and checked carefully whether any permanent morphological change has taken place. The mutants were selected for further analysis, growth of mycelia, colour of hyphae and conidia etc. were considered while selection was made.

i) Growth test of different morphological mutants on SM, VM

Growth tests were made in sterilized petridishes containing sorbose minimal medium, Vogel's minimal medium. The plates were divided into 24 compartments by glass marking pencil. The 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.

Study of Mutagenic Effect of Leaf Extract of Tea (*Camelia sinensis* Linn O. Kuntze) on *Neurospor crassa.*

Preparation of solution of different concentration of Tea (*Camelia sinensis* Linn O. Kuntze)

Experiment was made to find out the mutagenic effect of tea leave extract on *Neurospora crassa* Tea extract of different density were prepared. 150 gm leaves of tea were taken. Washed 3 times with tap water and 3 times with distilled water. After air drying leaf were cutted into small pieces and grinded with 4 ml of distilled water in an electric grinder. The paste was filtrated with a sterilized cotton cloth and finally filtred with filter paper to get clean solution.

All extract namely 0.5, 1.0, 2.0, 4.0, 6.0 were prepared separately in sterilized test tube in refrigerator.

Effect of extract of tea on the growth of *Neurospora crassa*

For testing the effect of tea extract on the growth of *Neurospor crassa* with different concentration of tea solution were prepared. Different solution were taken separately on the petridish at the rate of 0.5 ml, 1.0 ml, 2.0 ml, 4.0 ml, 6.0 ml and only 10 ml of VM on a petridish. 10 ml of molten VM was added in each petridish and rotated gently for uniform mixing of solution with medium. When the medium became solid the centre of the petridish was marked and fresh culture of Ema was inoculated at that point with a sterilized needle.

All the petridishes were kept in incubator at 25^0C after 18, 24, 30 and 36 hours the radial growth of Ema of each petridish was measured in cm.

Treating of Ema with leaf extract of tea

a) Obtaining of fresh culture

To obtaining fresh culture Ema was cultured 4 times after 4 days intervals. In each case 5 days of culture was used for treating conidia.

b) Sterilization

Al the media, essential elements and instruments were sterilized in the autoclave at 120^0c under 15lb pressures for 30 minutes. The inoculation chamber, needle, centrifuge machine etc. were sterilized with rectified spirit.

c) Centrifugation

10 ml leaf extract of the concentration were taken into 2 centrifuge tubes. 1 loop of conidia of Ema was taken into each tube and was shaken for homogenous solution. The solution was centrifuged with the help of a centrifuge machine for 15-20 minutes.

d) Filtration

After centrifugation the solution above conidia was poured out from the centrifuge tube. 10 ml of sterilized distilled water was added in the centrifuge tube and centrifuged for 3 minutes then the distilled water was poured out. The same procedure was repeated 2 times.

e) Preparation of suspension with treated conidia

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

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i) Growth test of different morphological mutants on SM, VM

Growth tests were made in sterilized petridishes containing sorbose minimal medium, Vogel's minimal medium. The plates were divided into 24 compartments by glass marking pencil. The 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.

Study of linear growth

Linear growths of selected mutants were measured in race tube, made of long Pyrex glass tube. The horizontal portion was kept 36 cm long and inner diameter of about 15 mm. The two sides which were bent up one at angle of 45^0 and opposite side at 90 angles with a lower projection of 1cm. These tubes were held up right on a flat surface with 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 growths of the tube were measure in cm at 6 hours interval.

Study of mycelial growth

It was done in liquid Vogel's minimal medium. Selected mutants and Ema was inoculated in the medium of the flask. Each flask contained 50 ml medium. To prevent the formation of conidia, flasks were shaken continuously.

Weight of the empty filter papers was taken when the culture was 96 housr old; flasks with culture were boiled in water for 2 minutes and were filtred with filter paper of known weight. The filter papers with mycelia were dried in an oven and the weights of filter papers with culture were taken. Those were done with control.

Induction of Mutation with UV ray (254nm)

Filtration Enrichment Method:

The wild type strain of *N. crassa*, Ema, (5297) was subcultured several times to make the culture fresh. Conidial suspension from 5 day old cultures were prepared by transferring 2 loopfuls of conidia into a tube containing about 10 ml sterilized distdtilled water. The suspension was filtered through a sterilized cotton filter to eliminate the mycelial debris and conidial lumps.

About 10 ml of the filtered conidial suspension was taken into each small sterilized Petri dish and then two such Petridishes with conidia were irradiated with ultra violet ray. The irradiated conidia in the petridishes were kept in dark for 2 hours. After that they were inoculated into 4 large (1.5′×9″) tubes each containing 25 ml VM liquid medium The inoculated tubes were placed in a water tank in which the temperature was maintained with the help of a circotherm unit at 25^0C. Then filtered air was pumped through a Pasteur pipetted inserted into each culture tube to secure continued suspension of the conidia. To secure continued suspension of the conidia and to keep the germinating conidia separated from each other, it was found necessary to have the orfice of the Pasteur pipette very close to the bottom of the tube.

After every six hours. The irradiated conidia were filtered through a cotton filter. After each filtration some fresh liquid VM was added into the tube and it was then put back into the controlled temperature tank. This procedure was continued for 3 days. Afeter the final filtration, same amount of the conidial suspension were plated in SM, Supplemented with 30 mg tryptophan, leucine, lysine, arginine, histidine and adenine per 100 ml. The plates were incubated at 25^0C and cheeked for the growth of colonies up to 7 days. Well separated single conidial colonies were isolated in VM tubes supplemented with those amino acids.

The tubes were incubated at 25^{0}C for 5 days. These single conidial cultures were then tested on SM the nongrowing ones on SM were selected as mutants

SELECTION OF SOME MORPHOLOGICAL MUTANTS

Mutants from different groups were subcultured three times and observation was taken whether there was any change in their characters. The mutants which retain their characters every time were finally selected for further studies such as linear growth, Radial growth, mycelial growth etc.

STUDY OF LINEAR GROWTH

Linear growths of selected mutants were measured in race tube, made of long pyres 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 45^0 and opposite side at 90^{0}angle with a lower projection of 1cm. Those tubes were held up right on a flat surface with 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 growths of the tube were measured in cm at 12 hours interval.

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 weights of filter papers with culture were taken. Those were done with control.

Test of Single pollutants (As and Pb)

There are so many pollutants which are causing environmental pollution. Here As and Pb were selected as Single pollutants. The fast growing mutant vigorous was selected to study bisorption capacity of the fungus with these pollutants.

Preparation of As solution

At first 100 ml water was added with 0.1 mg AsO_3 and 1 drop of NaOH. Then biomass was added with this solution. After 24 hours biomass was took off from the As contaminated water. Then the concentration of As was measured with the help of Atomic Absorption Spectrophotometer.

Preparation of Pb solution

At first 100 ml water was added with 0.1 mg lead acetate and 1 drop of HNO_3. Then biomass was added with this solution. After 24 hours biomass was took off from the Pb contaminated water. Then the concentration of Pb was measured with the help of Atomic Absorption Spectrophotometer.

Test of Mixed pollutants (Dye & tannery pollutants)

So for the study of removal of environmental pollutants, Tannery and Dye pollutants were selected as mixed pollutants. Tannery and Dye pollutants contain many chemicals and other substances. These were collected from Tannery of Hazaribag, Dhaka and Laundry of Jagannath Hall, Dhaka University respectively. Then the biomass of selected mutant and wild type (Ema) were added with the polluted water and observed after 3,6 and 9 hours if there be any change. The biomass were again dried in the 58^0C incubator for 24 hours and weight of biomass were recorded. All the experiments were done with control.

EXPERIMENT AND RESULTS

Fig-1: *Neurospora crassa* wild type Ema and EmA

Fig-1: *Neurospora crassa* wild type Ema and EmA

EXPERIMENT AND RESULTS

Study of mutagenic effect of leaf extract of papaya (*Carica papaya* Linn) on *Neurospora crassa.*

Different concentrations of papaya solution were used to test the mutugenic effect on *Neurospora crassa*. Fixed concentration had appropriate mutagenic effect on *N. crassa.*

Preparation of different extract of papaya leaves

Different concentrations of leaf extract of papaya solution were prepared.

Table-1

Different concentration of papaya solution

Total amount of leaf	Name of the solution	Amount of Extract
120 gm	0.5	0.5ml
	1.0	1 ml
	2.0	2 ml
	4.0	4ml

Effect of leaf extract of papay on the growth of *Neurospora crassa.*

For testing the effect, different solution was taken separately on the sterilized petridish at the rate of 0.5 ml, 1 ml, 2ml and 4 ml. 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^{0}C and measured the radial growth after 18, 24, 30 and 36 hours. Following were the results (Table-2).

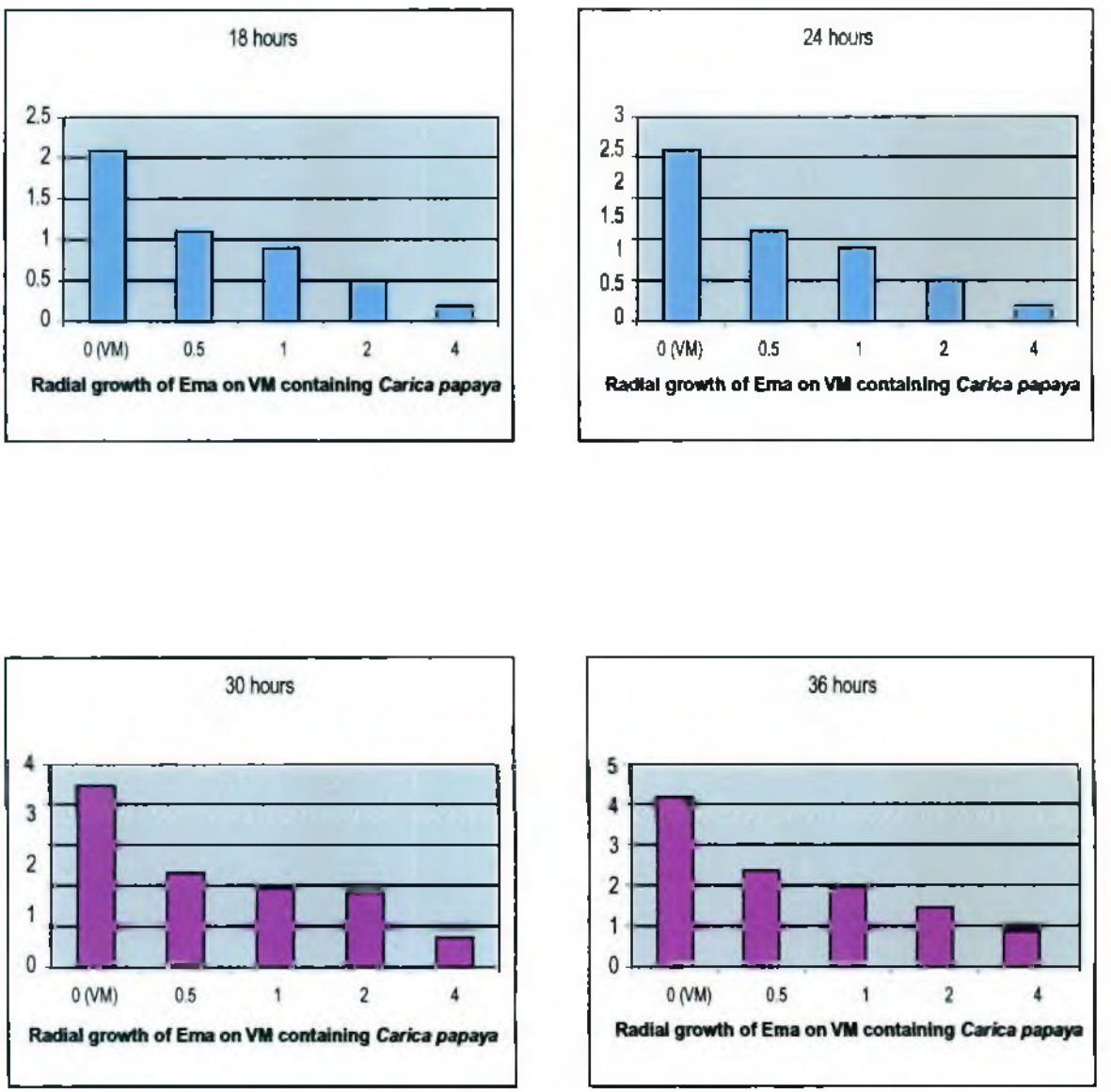

Fig-2: Radial growth of *Carica papaya* Linn.

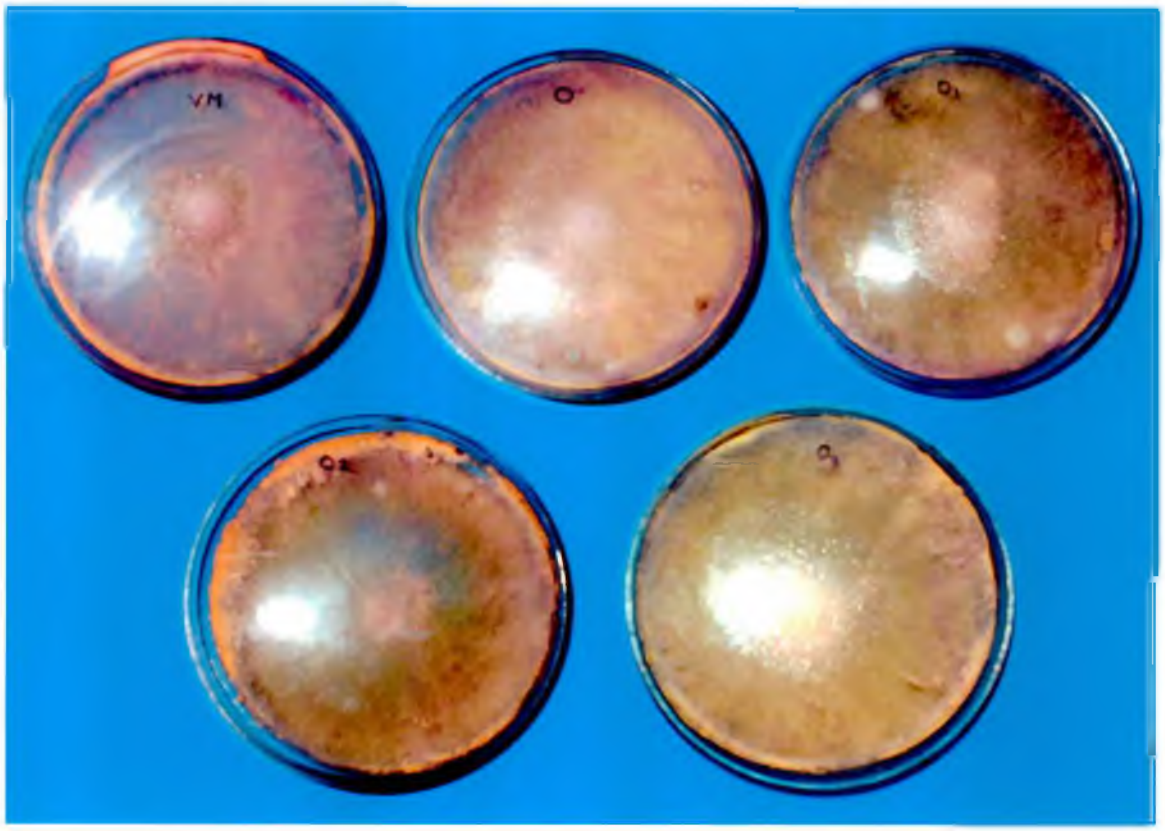

Fig-3: Radial growth of *Carica papaya* Linn.

Table-2

Radial growth of Ema on VM containing leaf extract of papaya (*Carica papaya* Linn)

Concentration of the solution	Time of the observation after following hours in CM			
	18 hours	24 hours	30 hours	36 hours
0 (VM)	2.1	2.4	3.6	4.3
0.5	1.1	1.3	1.9	2.5
1.0	.9	1.0	1.6	2.1
2.0	.5	.7	1.5	1.6
4.0	.2	.3	.6	1

Treating of Ema with leaf extract of Papaya

The treating time was 15 minutes.

Classification of papaya induced mutant.

Group A: *Vigorous*

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

501, 589, 600, 711, 719, 749, 811, 826, 876, 901, 913.

Group B: *Cauliflower*

Checked growth, conidia form cauliflower like structure. The following 3 mutants fall in this group.

121, 131, 139.

Group C: *Fluffy*

Mycelia are colourless, conidia are absent or very few in number, mycelia are cottony and growth is vigorous. The following 7 mutants fall into this group.

312, 327, 351, 378, 804, 809, 825.

Group D: *Fluffy band*

Colourless mycelia from a band shaped structure at the top, steady in the tube. The following 3 mutants fall into the group

451, 486, 489.

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 includes the following 39 mutants.

189, 197, 205, 211, 228, 239, 247, 249, 252, 253, 258, 269, 271, 279, 281, 285, 289, 292, 293, 297, 300, 303, 304, 307, 309, 310, 333, 357, 389, 401, 484, 494, 496, 532, 582, 599, 601, 605, 617

Table-3

Classification and Nomenclature of papaya induced mutants.

Name of the group	Characteristics	No of mutant	Name of the mutant
A	Huge conidial growth that touches the plug	11	vigorous
B	Checked growth, conidia form cauliflower link structure	3	cauliflower
C	Profuge mycelial growth	7	Fluffy
D	Densed colourless mycelial band from at tip	3	Fluffy band
E	Conidia formed a band at the tip of the growth	39	Conidial band

Fig-4: *Carica papaya* Linn induced Mutants & Ema.

Study of mutagenic effects of fruit extract of bitter gourd (*Momordica charantia* Linn) on *Neurospora crassa*.

Different concentrations of bitter gourd sohution were used to test the mutagenic effect on *Neurospora crassa*. Fixed concentrations had appropriate mutagenic effect on *N. crassa*.

Preparation of different cocentratiou of bitter gourd fruit extract.

Different concentrations of fruit extract of bitter gourd solution were prepared.

Table - 4

Different concentration of solution

Total amount of fruit	Name of the solution	Amount of extract
60gm	0.5	5ml
	1.0	1ml
	2.0	2ml
	4.0	4ml

Effect of fruit extract of bitter gourd *(Momordica charantia* Linn*)* on the growth of *Neurospora crassa.*

For testing the effect different solution was taken separately on the sterilized petridish at the rate of 0.5ml, 1ml. 10ml of molten VM was added in each Petridish. When media became solid, Ema was inoculated at the center of the media. Plates were incubated at 25^0c and measured the radial growth after 18, 24, 30 and 36 hours. Following were the results (Table-5).

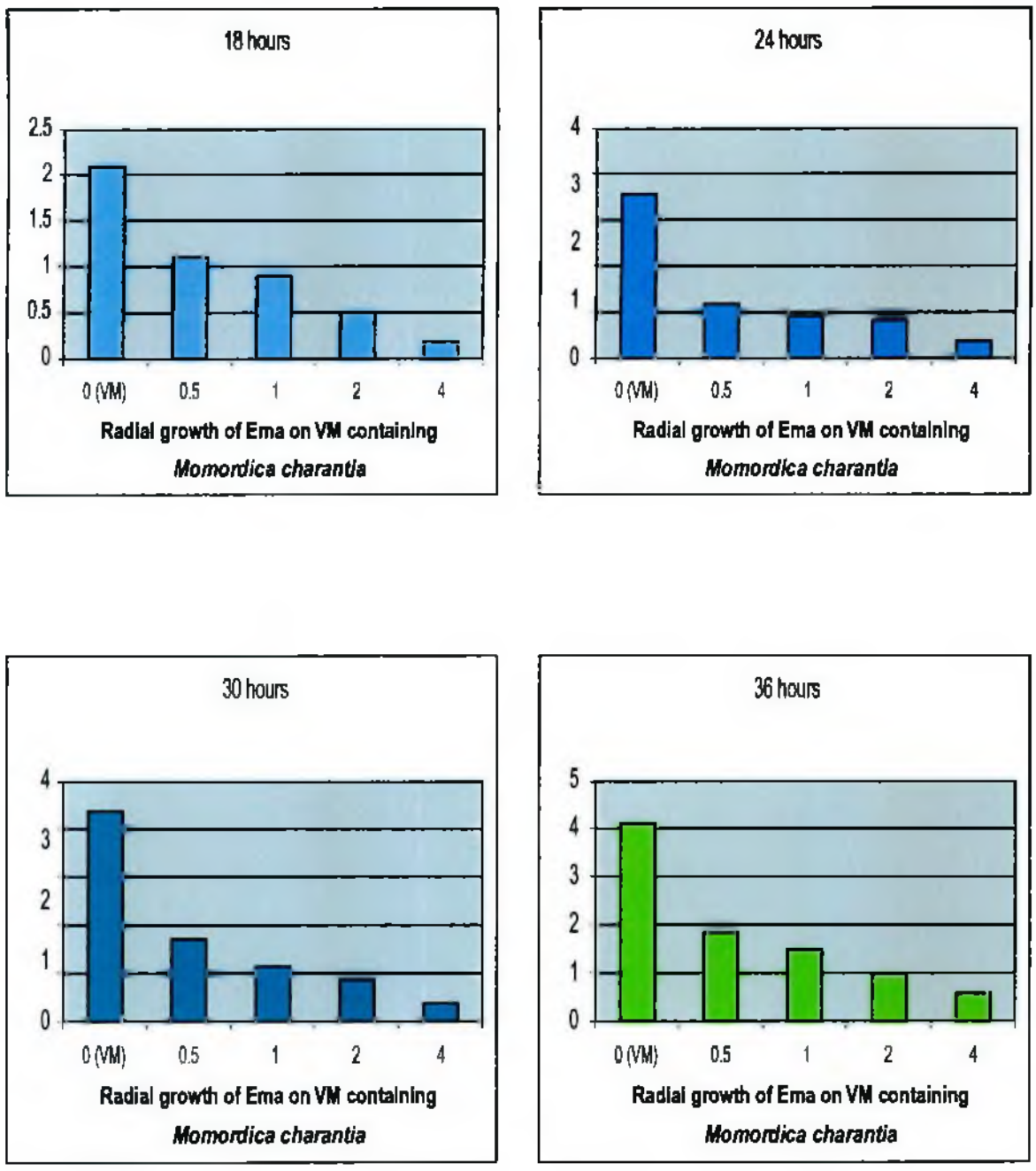

Fig-5: Radial growth of *Momordica charantia* Linn.

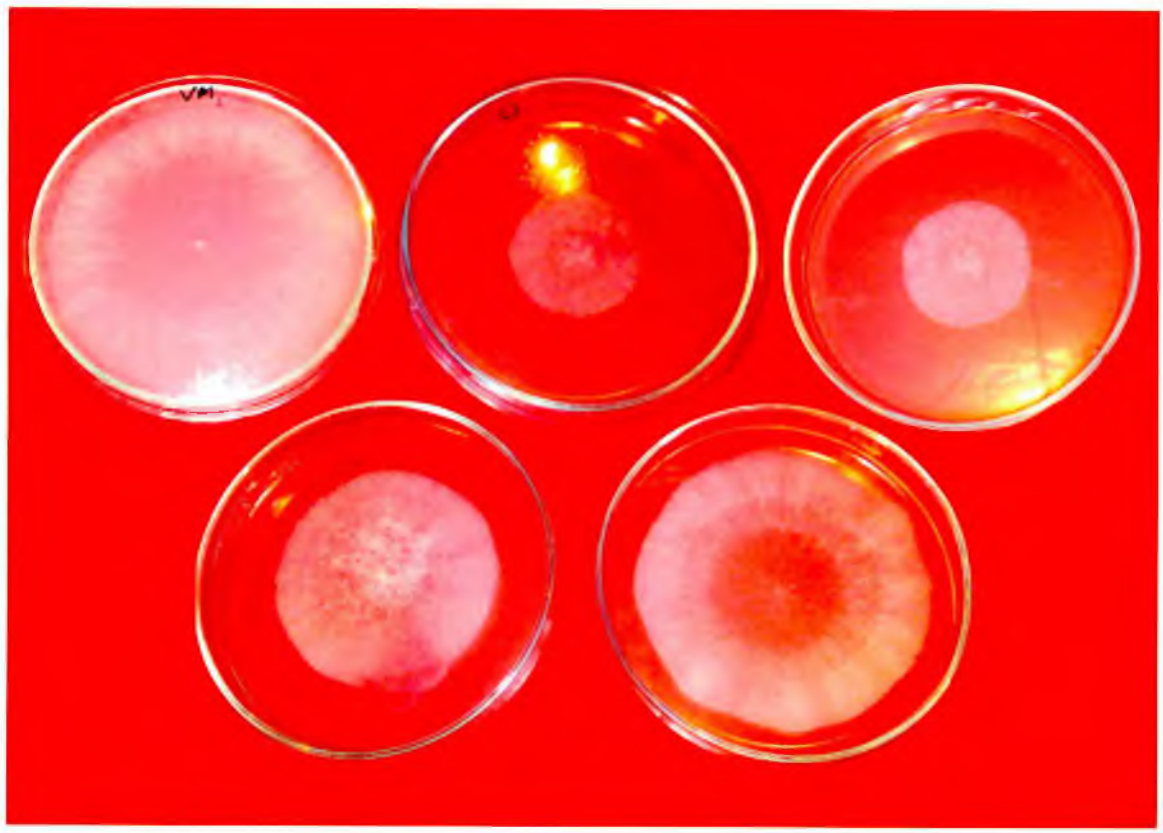

Fig-6: Radial growth of *Momordica charantia* Linn.

Table – 5

Radial growth of Ema on VM containing fruit of bitter gourd (*Momordica charantia* Linn)

Concentration of the solution	Time of the observation after following hours in cm			
	18 hours	24 hours	30 hours	36 hours
0 (VM)	2.2	3.0	3.6	4
0.5	.8	1.0	1.5	1.8
1.0	.6	.8	1.0	1.5
2.0	.6	.7	.8	1.0
4.0	.3	.3	.4	.6

Treating of Ema with fruit extract of bitter gourd *Momordica charantia* Linn)

The treating time was 15 minutes.

Classification of bitter gourd induced mutant

Group A: Dirty

Conidial lump scattered throughout the body of the culture tube. The following 7mutants full in this group.

49, 58, 61, 79, 86, 92

Group B: Ropy

Mycelia form rope like structure and conidia pink. The following 11 mutants fall in this group.

18, 26, 27, 31, 39, 43, 52, 103, 127, 401, 429.

Group C: Albino

Both mycelia and conidia are colourless.

The following 19 mutants fall into this group.

2, 8, 9, 13, 14, 17, 21, 29, 35, 40, 41, 42, 54, 56, 59, 82, 85, 86, 98.

Group D: Vigorous

Pink coloured profuse conidial growth which reaches up to the plug. The following 22 mutants fall into this group.

301, 309, 321, 339, 347, 378, 379, 382, 387, 399, 405, 426, 435, 439, 444, 447, 470, 482, 487, 491, 496, 499.

Fig-7: *Momordica charantia* Linn induced Mutants & Ema.

Table – 6

Name of the group	Characteristics	No. of mutant	Name of the mutant
A	Conidial lump scattered throughout the body of the culture tube.	6	Dirty
B	Mycelia form rope like structure and conidia pink.	11	Ropy
C	Both mycelia and condia are colourless	19	Albino
D	Profuse conidial and mycelia growth reach the plug.	22	Vigorous

Study of mutagenic effect of leaf extract of tea (*Camelia sinensis* Linn O. kuntze) on *Neurospora crassa*.

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

Preparation of different extract of tea leaves.

Different counteractions of leaf extract of tea solution were prepared.

Table – 7

Different concentration of solution

Total amount of leaf	Name of the solution	Amount of extract
150gm	0.5	5ml
	1.0	1ml
	2.0	2ml
	4.0	4ml
	6.0	6ml

Effects of leaf extract of tea on the growth of *Neurospora crassa*.

For testing the effect, different solution was taken separately on the sterilized petridish at the rate of 0.5ml, 1ml, 2ml, 4ml and 6ml. 10ml of molten VM was added in each petridish. When media became solid Ema was inoculate at the centre of the media. Plates were incubated at 25^{0}c and measured the radial growth after 18, 24, 30 and 36 hours. Following were the results. (Table- 8).

Table – 8

Radial growth of Ema on VM containing leaf extract of Tea (*Camelia sinensis* Linn. O.Kuntze)

Concentration of the solution	Time of the observation after following hours in cm			
	18 hours	24 hours	30 hours	36 hours
0 (VM)	2.0	2.6	3.3	4.9
0.5	1.6	2.1	2.8	3.0
1.0	1.4	1.8	2.4	3.0
2.0	1.0	1.3	1.7	2.3
4.0	.2	.4	.6	1.3
6.0	.1	.3	.7	1.0

Treating of Ema with leaf extract of Tea (*Camelia sinensis* Linn. O. kuntze)

The treating time was 15 minutes.

Classification of tea induced mutant

Group A: conidial band

Dense conidial growth form a band shaped structure at the top. Colour of the conidia is pink or orange. This group includes the following 7mutants.

28, 31, 45, 67, 69, 81, 83.

Group B: Plug

This mycelia growth inside the tube deep pink conidia formed outside the tube, conidia around the plug. 5 mutants full under this group

17, 24, 26, 52, 61.

Group C: 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 group.

21, 23, 34, 39, 47, 58, 60, 79, 86, 92, 150.

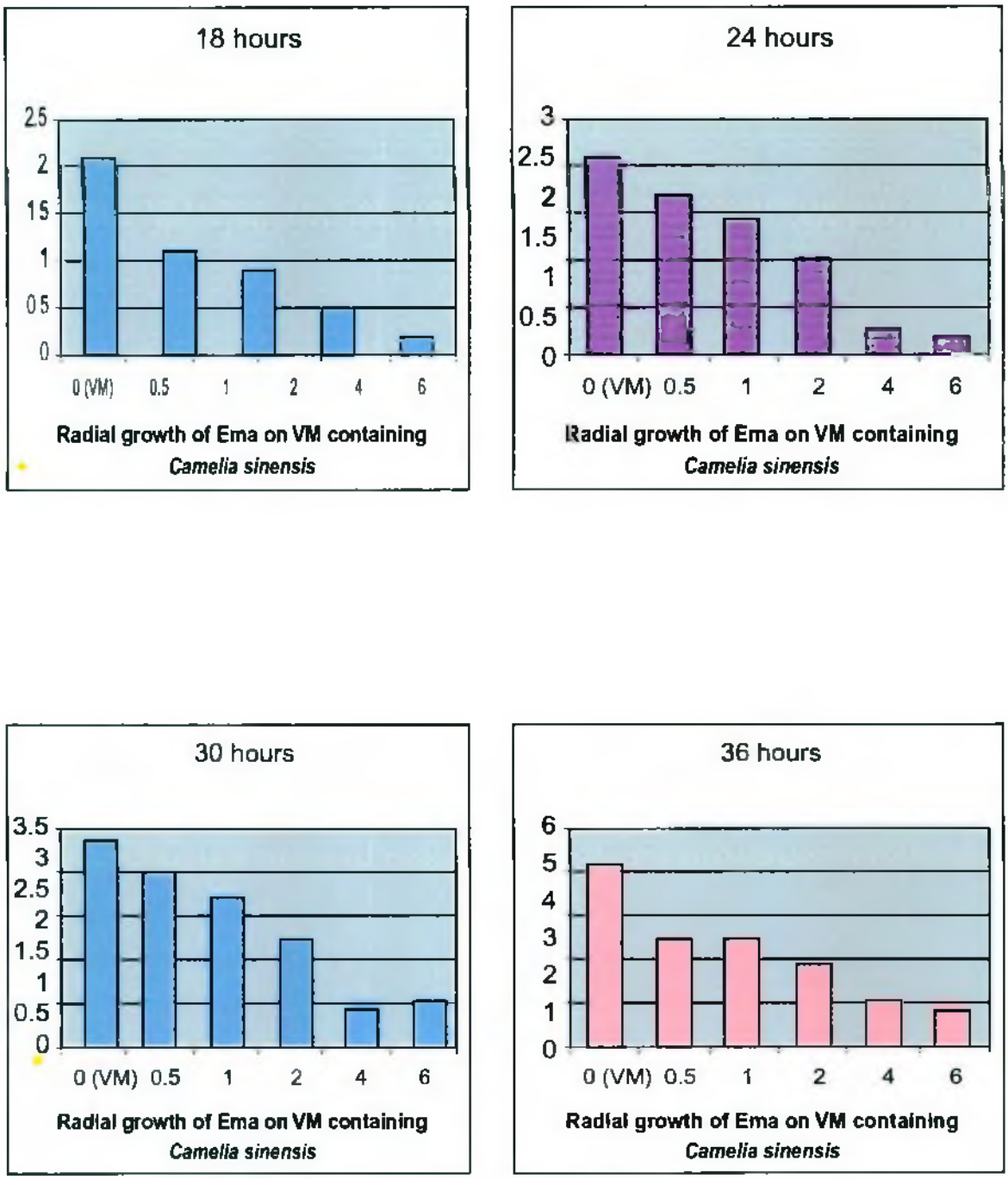

Fig-8: Radial growth of *Camelia sinensis* Linn.

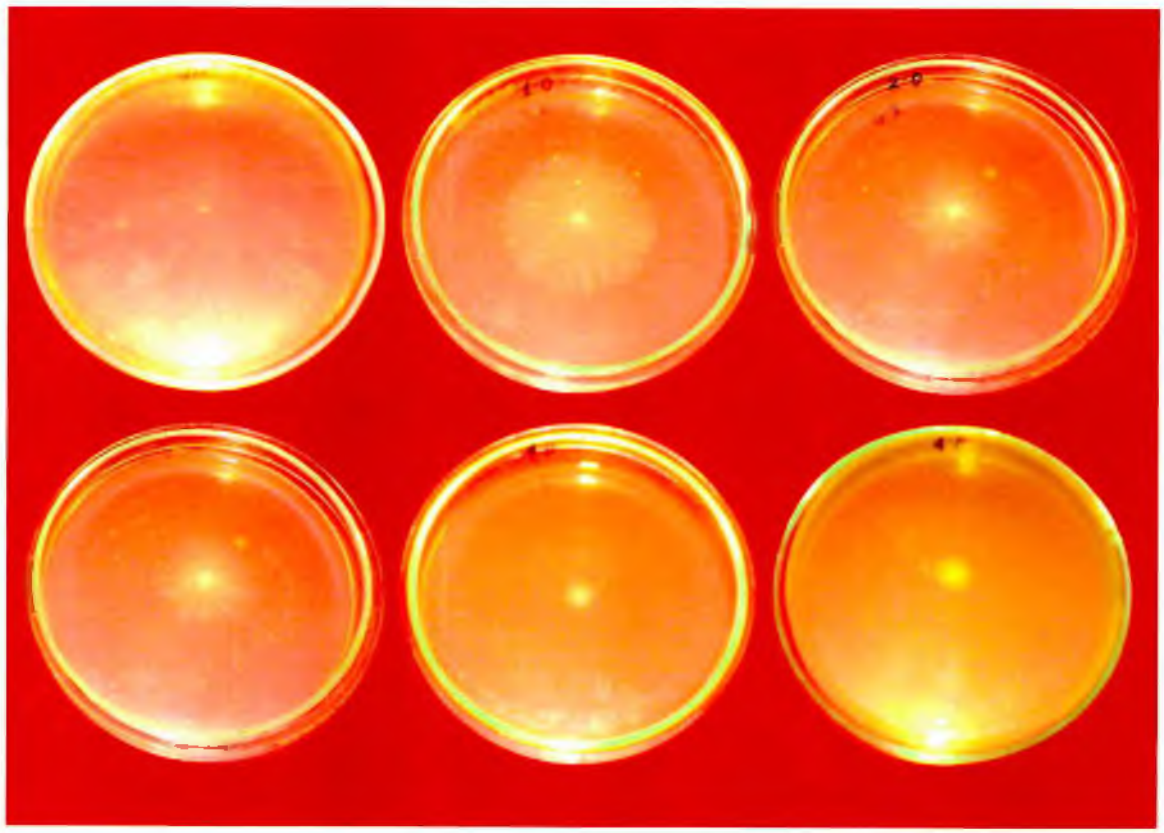

Fig-9: Radial growth of *Camelia sinensis* Linn.

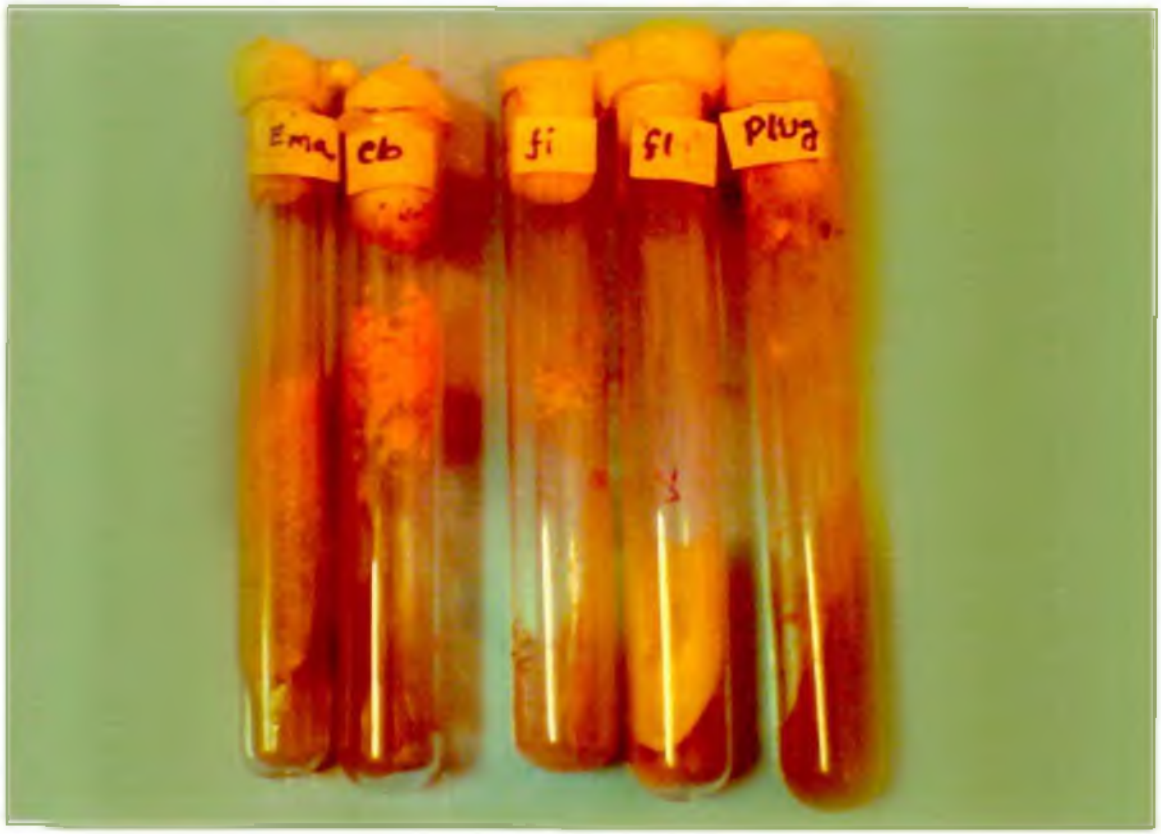

Fig-10: *Camelia sinensis* Linn induced Mutants & Ema.

Group D: Fissure

Very checked growth lie on media, conidia present and light pink in colour, upper portion of the media was separated by a breakage.

The following 2 mutants fall into this group.

22, 98.

Table – 9

Classification and nomenclature of tea induced mutant

Name of the group	Characteristics	No. of mutant	Name of the mutant
A	Conidia form a band like structure at the top	7	Conidial band
B	Conidia formed outside the plug	5	Plug
C	Profuse mycelia growth, no conidia formed	11	Fluffy
D	Very cheeked growth lies on the media. Light pink conidia and media were broken.	2	Fissure

Selection of some mutants for further study

From these 3 mutations 10 types of mutants were obtained. From there a good number of mutants were selected for study of mycelial weight and linear growth.

Table – 10

Selected mutants for further study

Serial No.	Mutant Number	Name of the mutant
1.	501, 589, 600, 711, 719	Vigorous
2	121, 131, 139	Cauliflower
3	312, 351, 804	Fluffy
4	451, 486	Fluffy band
5	300, 357	Conidial band
6	103, 127	Ropy
7	2, 9, 13	Albino
8	17, 24, 26	Plug

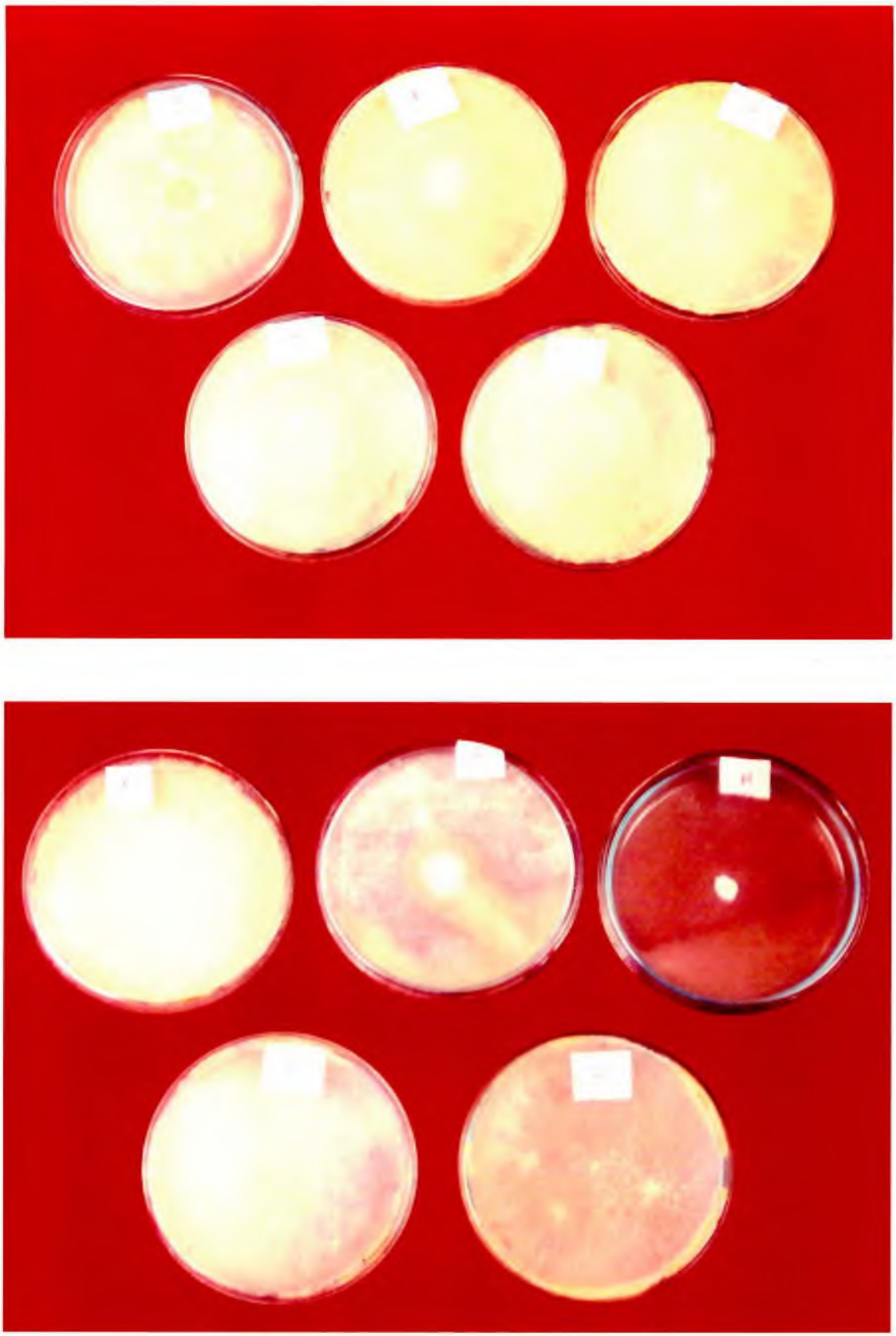

Fig-11: Colonial growth of some Mutants & Ema.

Table -11

Mycelial weight of some selected mutants and Ema

Name of the cultures	Weight of the empty filter paper (g)	Weight of filter paper with dried mycelia (g)	Weight of the dried mycelia (g)	Mean (g)
Vigorous	1.005	2.0736	1.0686	1.0686
	1.005	2.0737	1.0687	
Cauliflower	1.003	1.058	0.055	0.051
	1.002	1.049	0.047	
Fluffy	1.002	1.4725	0.4705	0.46475
	1.003	1.4620	0.459	
Fluffy band	1.003	1.4083	0.4053	0.40375
	1.003	1.4052	0.4022	
Conidial band	1.001	1.3412	0.3402	0.3745
	1.002	1.4108	0.4088	
Ropy	1.002	1.256	0.256	0.2445
	1.002	1.235	0.233	
Albino	1.002	1.4303	0.4273	0.4272
	1.003	1.4301	0.4271	
Plug	1.002	1.4901	0.4881	0.48795
	1.001	1.4888	0.4078	
Ema	1.002	1.4269	0.4249	0.42945
	1.003	1.4370	0.434	

Fig-12: Mycelial growth of Ema and Mutants in shaker.

Table-12

Linear growth of Ema and some selected mutants

Name of the cultures	Growth in cm after the following hours.											
	24	Mean	30	Mean	36	Mean	42	Mean	48	Mean	54	Mean
Vigorous	4.6	4.7	5.7	5.85	10.0	10.4	14.0	13.95	16.0	16.35	19.8	19.65
	4.8		6.0		10.8		13.7		16.7		19.5	
Cauliflower	1.3	1.25	1.7	1.85	3.6	3.75	5.9	5.8	6.6	6.55	7.1	7
	1.2		2.0		3.9		5.7		6.5		6.9	
Fluffy	3.5	3.65	6.9	6.75	8.0	8.35	9.0	9.3	11.7	11.65	16.0	16.05
	3.8		6.6		8.7		9.6		11.6		16.1	
Fluffy band	2.3	2.5	3.5	3.6	3.9	4.05	5.0	5.1	6.7	6.55	7.9	7.85
	2.7		3.7		4.2		5.2		6.4		8.9	
Conidial band	3.4	3.5	5.1	5.2	7.9	7.75	10.9	10.85	12.9	13	14.1	14
	3.6		5.3		7.6		10.8		13.1		13.9	
Ropy	1.7	1.6	1.9	1.95	2.6	2.7	3.7	3.55	4.0	3.95	5.7	5.8
	1.4		2.0		2.8		3.4		3.9		5.9	
Albino	1.0	1.15	1.7	1.6	1.8	1.85	2.4	2.2	2.6	2.7	3.1	3.15
	1.3		1.5		1.9		2.0		2.8		3.2	
Plug	3.9	3.95	4.6	4.7	9.0	8.95	12.0	11.95	14.1	14.15	16.2	16.25
	4.0		4.8		8.9		11.2		14.2		16.3	
Ema	1.9	1.85	3.9	3.75	5.9	5.95	7.0	6.9	7.6	7.8	8.0	8.05
	1.8		3.6		6.0		6.8		8.0		8.1	

Name of the cultures	Growth in cm after the following hours.											
	60	Mean	66	Mean	72	Mean	78	Mean	84	Mean	90	Mean
Vigorous	24.1	24	26.1	26.1	28.0	27.95	30.9	30.65	36.5	36.55	39.9	40
	23.9		26.2		27.9		30.6		36.6		40.1	
Cauliflower	7.6	7.5	8.1	8.25	8.6	8.75	9.2	9.35	10.2	10.45	13.5	13.45
	7.4		8.4		8.9		9.5		10.7		13.4	
Fluffy	18.1	18.25	22.6	22.65	24.0	24.15	26.9	26.8	32.5	32.45	35.1	35.05
	18.4		22.7		24.3		26.7		32.4		35.0	
Fluffy band	9.5	9.7	12.6	12.7	15.1	15.05	17.9	17.95	20.0	20.05	23.2	23.05
	9.9		12.8		15.0		18.0		20.1		22.9	
Conidial band	15.1	15.3	17.2	17.35	21.1	21.05	23.0	23.1	27.1	27.25	28.5	28.7
	15.6		17.5		21.0		23.2		27.4		28.9	
Ropy	6.5	6.7	8.2	8.1	10.6	10.75	13.1	13.4	15.9	15.85	18.1	8.05
	6.9		8.0		10.9		12.9		15.8		18.0	
Albino	4.1	4.15	5.8	5.85	6.0	6.25	7.2	7.1	8.0	8.05	8.9	8.95
	4.2		5.9		6.5		7.0		8.1		9.0	
Plug	21.1	21.05	23.0	23.05	25.9	25.85	27.7	27.65	33.0	32.95	35.9	35.75
	21.0		23.1		25.8		27.6		32.9		35.6	
Ema	10.6	10.65	15.6	12.7	14.1	13.15	14.5	14.55	15.1	15.05	15.6	15.65
	10.7		12.8		14.2		15.6		15.0		15.7	

Conclusion: It was observed from Table-11 that there was variation in the linear growth rate of selected mutants with Ema.

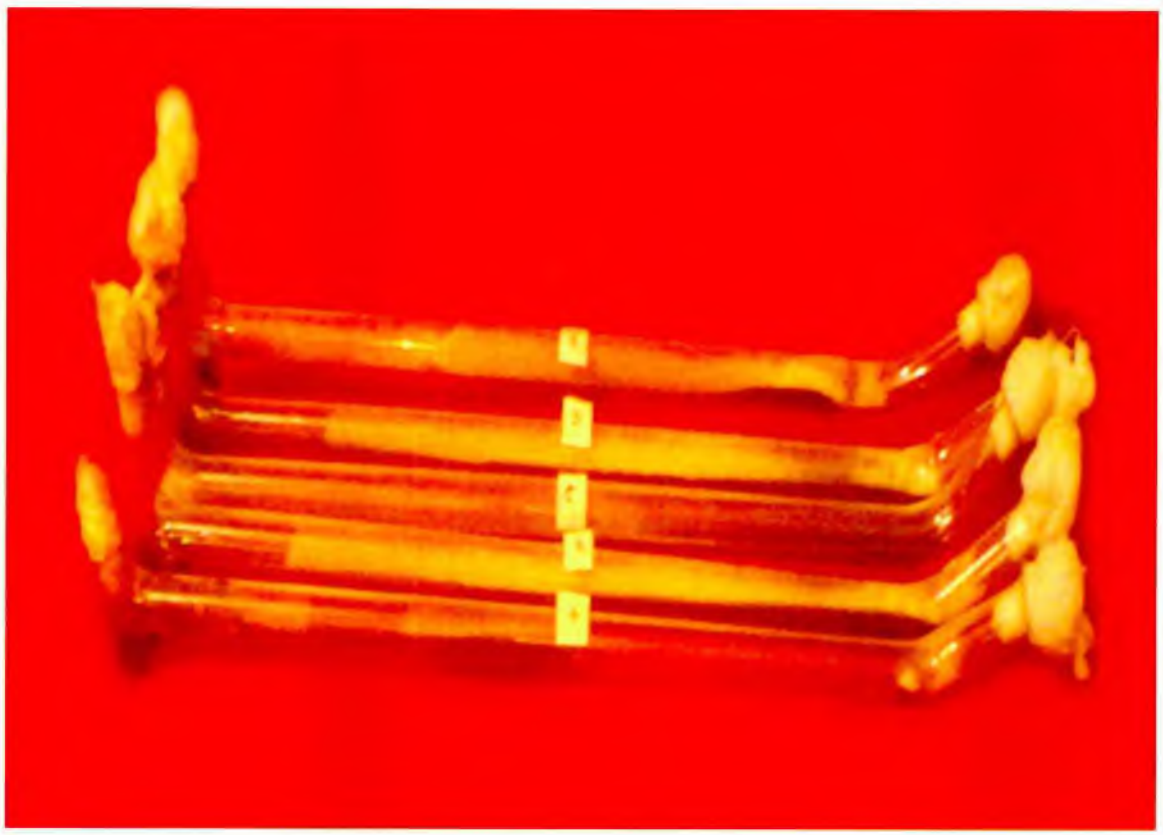

Fig-13: Liniar growth of some Mutants & Ema.

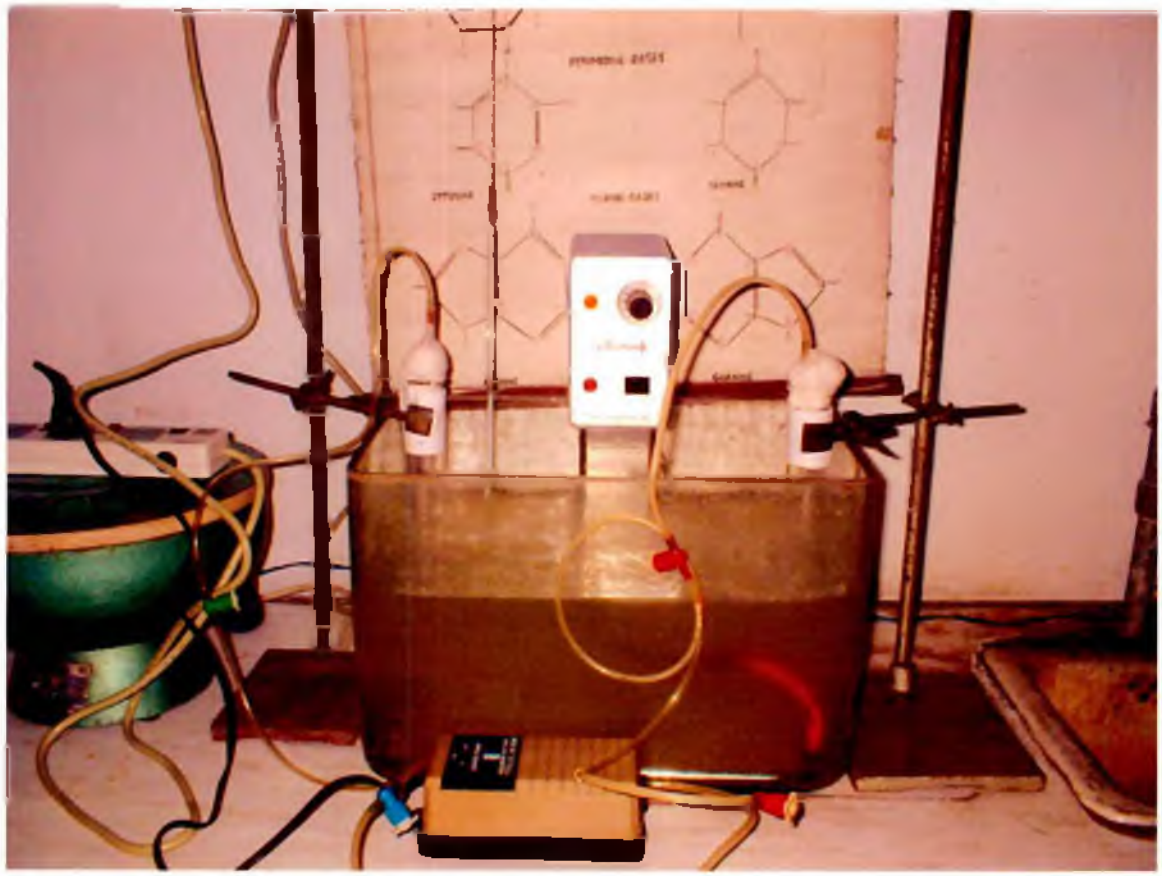

Fig-14: Apparatus showing filtration Enrichment method of UV Irradiation.

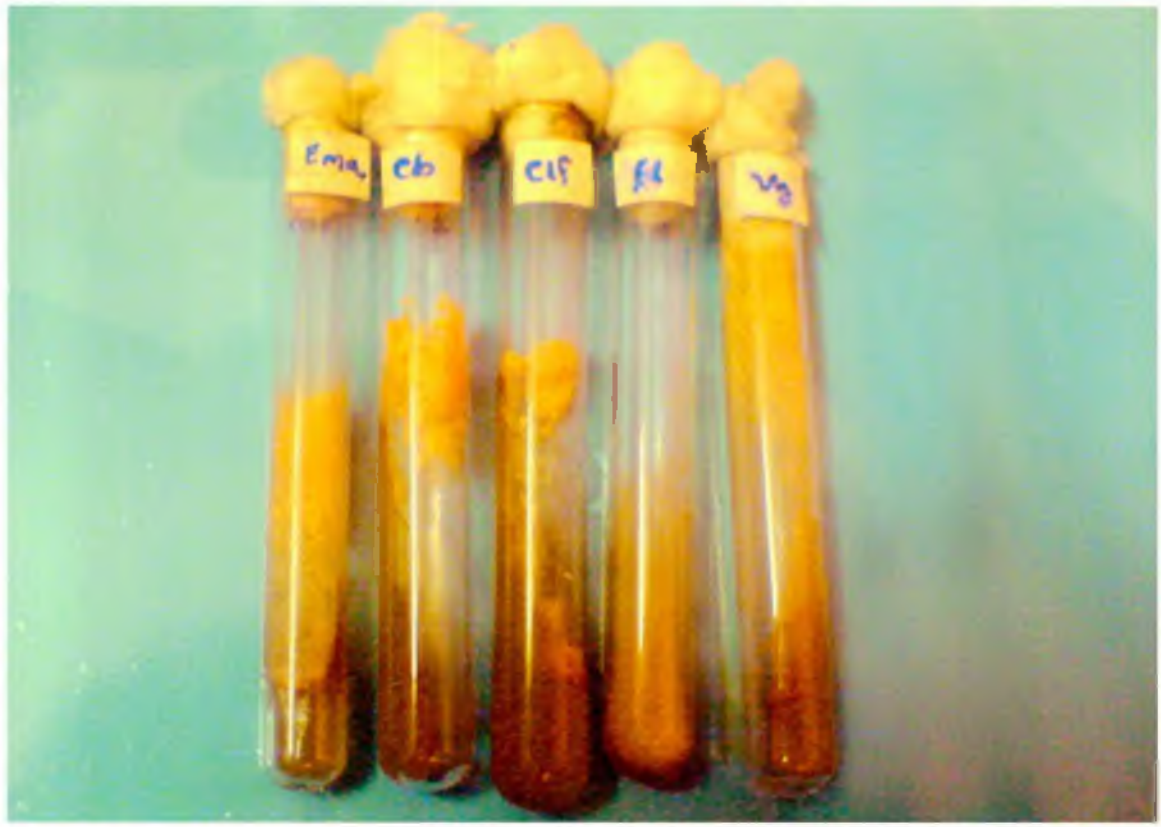

Fig-15: UV induced Mutants with Ema.

INDUCTION OF MUTATION BY UV IRRADIATION.

UV light of 254 nm ware length was used for treating conida and the treatment was done in the dark to avoid photo reactivating. The conidia of Ema were treated for 2 minute 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 7 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.

Table-13

Classification and nomenclature of UV induced mutants

Name of the group	Characteristics	Time of the treatment	No of mutant obtained	Name of mutant
A	Huge conidial growth that touches the plug	2'	5	vigorous
B	Profuse mycelial growth	2'	7	fluffy
C	conidia formed a band at the tip of the growth	2'	6	conidial band
D	Little restricted growth, mycelia and conidia formed a cauliflower like structures	2'	2	cauliflower

Mycelial weight of some selected UV induced mutants and Ema

It was done in Vogel's minimal liquid medium

Table-14

Mycelial weight of some UV induced mutants and Ema

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)
Vigorous	0.999	2.52	1.52	1.52
	0.999	2.52	1.52	
Fluffy	0.932	1.417	0.485	0.483
	0.999	1.479	0.481	
Conidial band	0.988	1.975	0.476	0.483
	0.987	1.475	0.491	
Cauliflower	0.892	1.002	0.110	0.1155
	0.890	1.011	0.121	
Ema	0.989	1.408	0.419	0.42
	0.987	1.408	0.421	

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

Linear growth of some selected UV induced mutants and Ema

Linear growths were measured in race tube.

Table-15

Linear growth of some UV induced mutants

Name of the cultures	Growth in CM after the following hours.											
	24	Mean	36	Mean	48	Mean	60	Mean	72	Mean	84	Mean
Vigorous	4.6	4.6	9.9	9.85	14.9	15	22.1	22.2	26.1	26.2	39.1	39.2
	4.6		9.8		15.1		22.3		26.3		39.3	
Fluffy	3.5	18.3	8.7	8.35	11.6	11.65	17.9	17.9	23.1	23	30.5	30.6
	3.4	3.45	8.0		11.7		17.9		22.9		30.7	
Conidial	3.4	3.5	7.2	7.3	12.6	12.6	14.9	14.9	19.9	19.85	7.1	27.1
band	3.6		7.4		12.6		14.9		19.8		7.1	
Cauliflowe	1.2	1.3	1.6	1.8	5.8	5.8	6.9	7	8.6	8.4	9.5	9.7
r	1.5		2.0		5.8		7.1		8.2		9.9	
Ema	2.2	2.2	6.1	6.05	11.0	11.0	15.8	15.7	18.5	18.5	24.0	24.0
	2.2		6.0		11.1		15.7		18.5		24.0	

Conclusion: It was observed from Table-15 that there was variation in the linear growth rate of selected mutants with Ema.

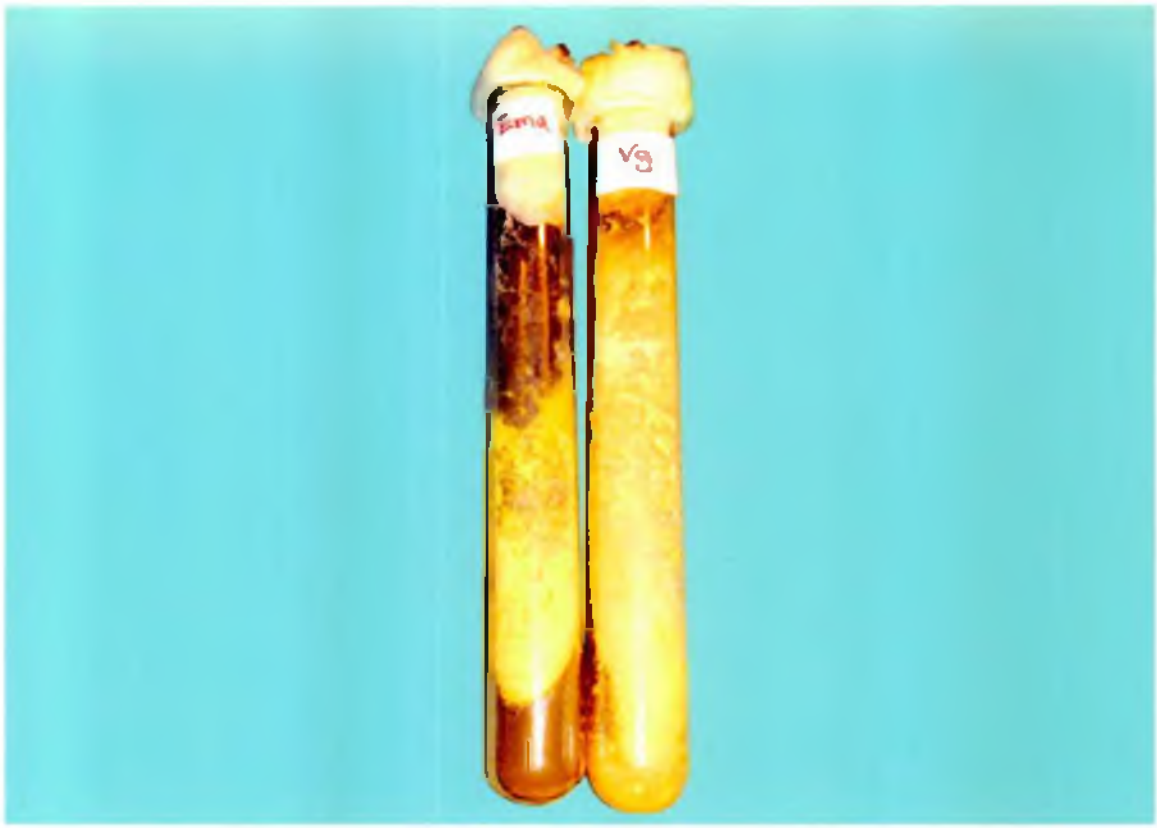

Fig-16: Ema & Vigorous Mutant.

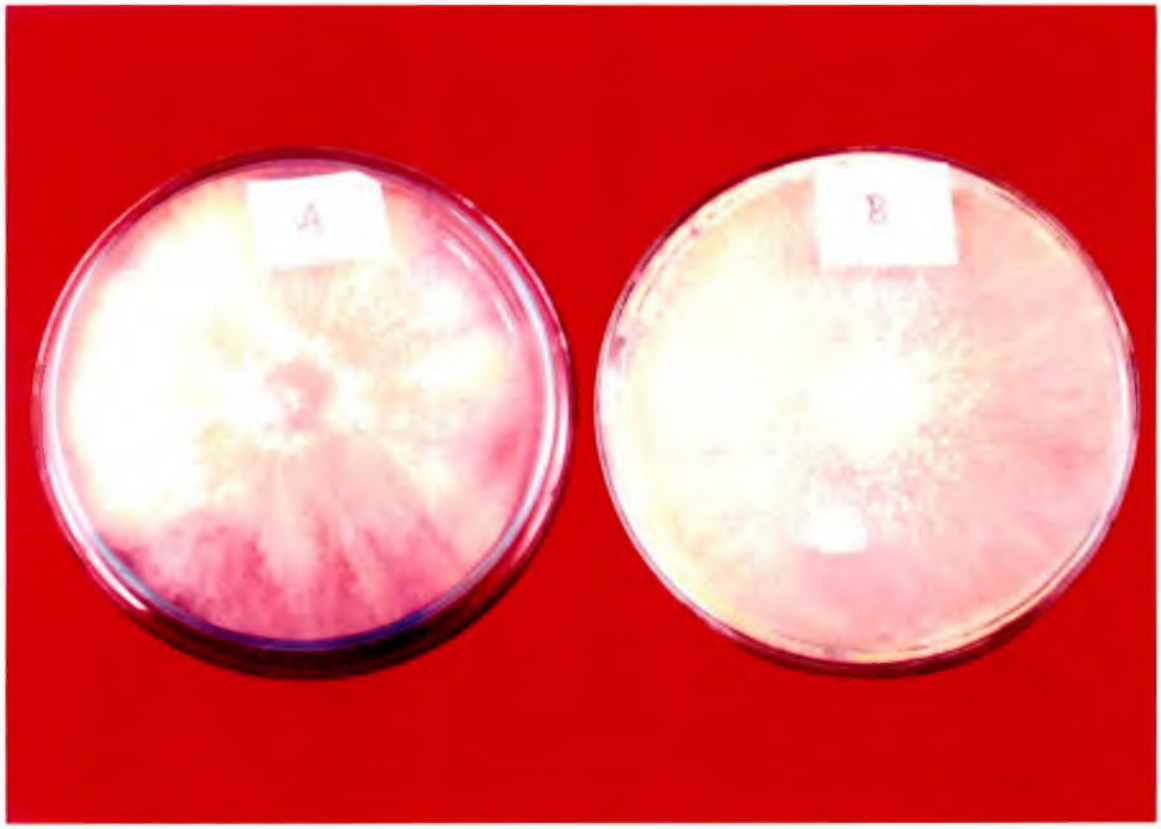

Fig-17: Colonial growth of *Vigorous* Mutant and Ema .

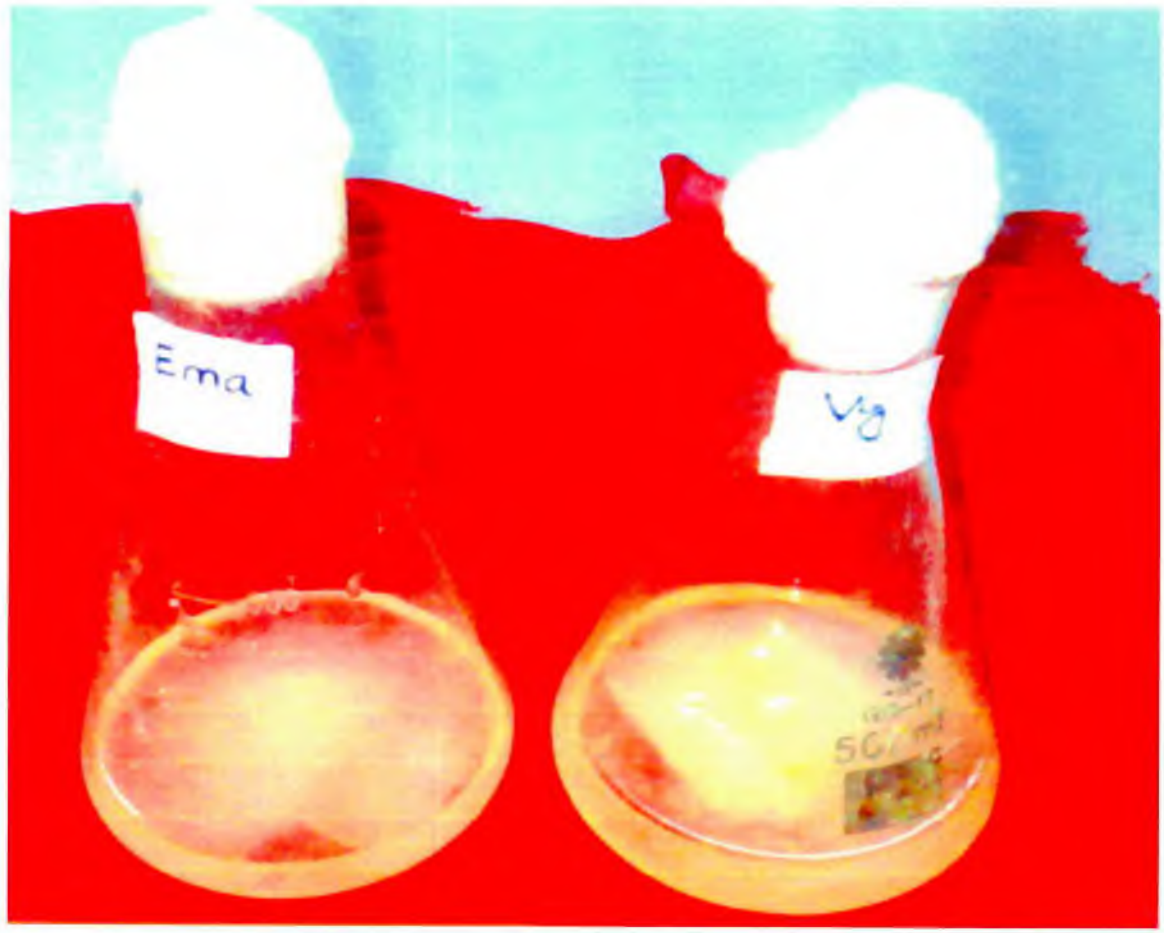

Fig-18: Mycelial growth of Ema and *Vigorous* Mutant.

Fig-19: Biomass of Ema.

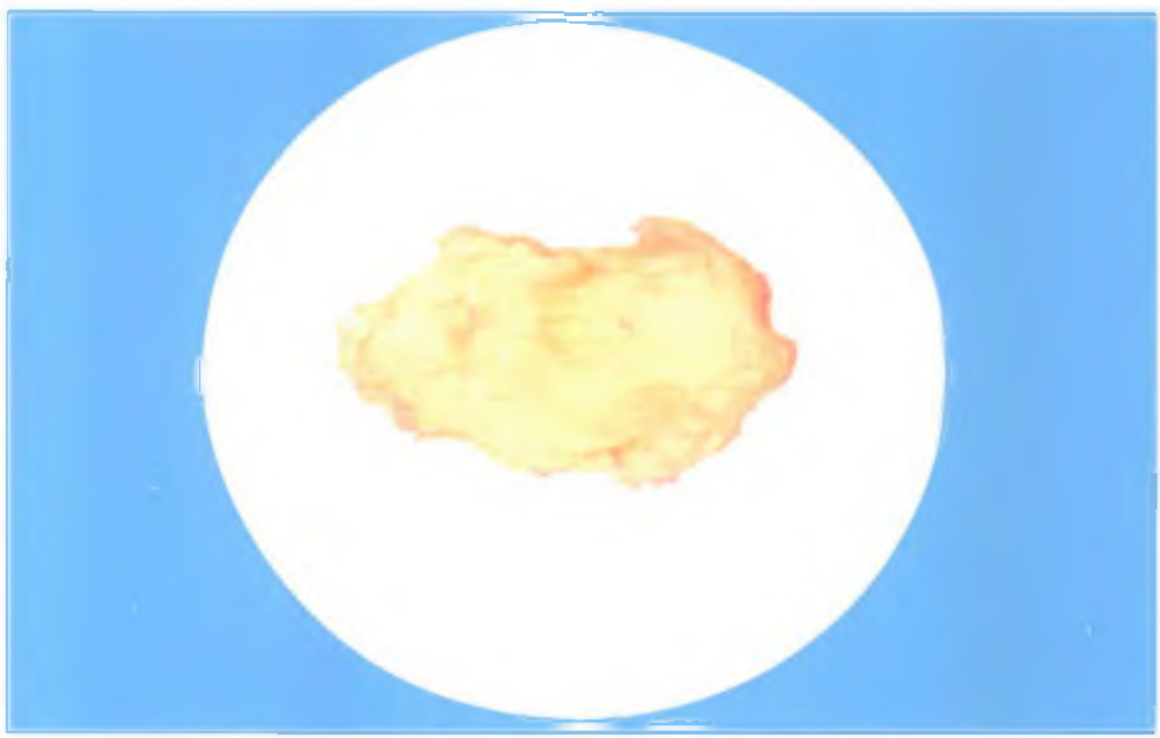

Fig-20: Biomass of *Vigorous* mutant.

Fig-21: Dye Pollutants of Jagannath Hall, Dhaka University.

Fig-22: Pond water of Jagannath Hall, Dhaka University.

Fig-23: Drain Containing Tannery palutants Hazaribag, Dhaka.

Study of biosorption capacity of *N. crassa* on Single and mixed pollutants

Result of Single pollutants (As and Pb)

At first 1 gm biomass was added with 100 ml of Arsenic polluted water and 100 ml of led polluted water. Both these water contain 0.1 mg As and lead respectively. After 24 hours, biomass was took off from both of those solutions and observed if there is any change took place.

Table-16

Biosorption capacity of *N. crassa* biomass with Single pollutants (As & Pb)

Name of the Pollutant	Amount of pollutant in 100 ml water (mg)	Observation after (hrs)	Quantity of Biomass		Absorption (g)
			Before experiment (g)	After experiment (g)	
Arsenic (As)	0.1	24	1	1	0
Lead (Pb)	0.1	24	1	1	0

Fig-24: Biomas with As & Pb.

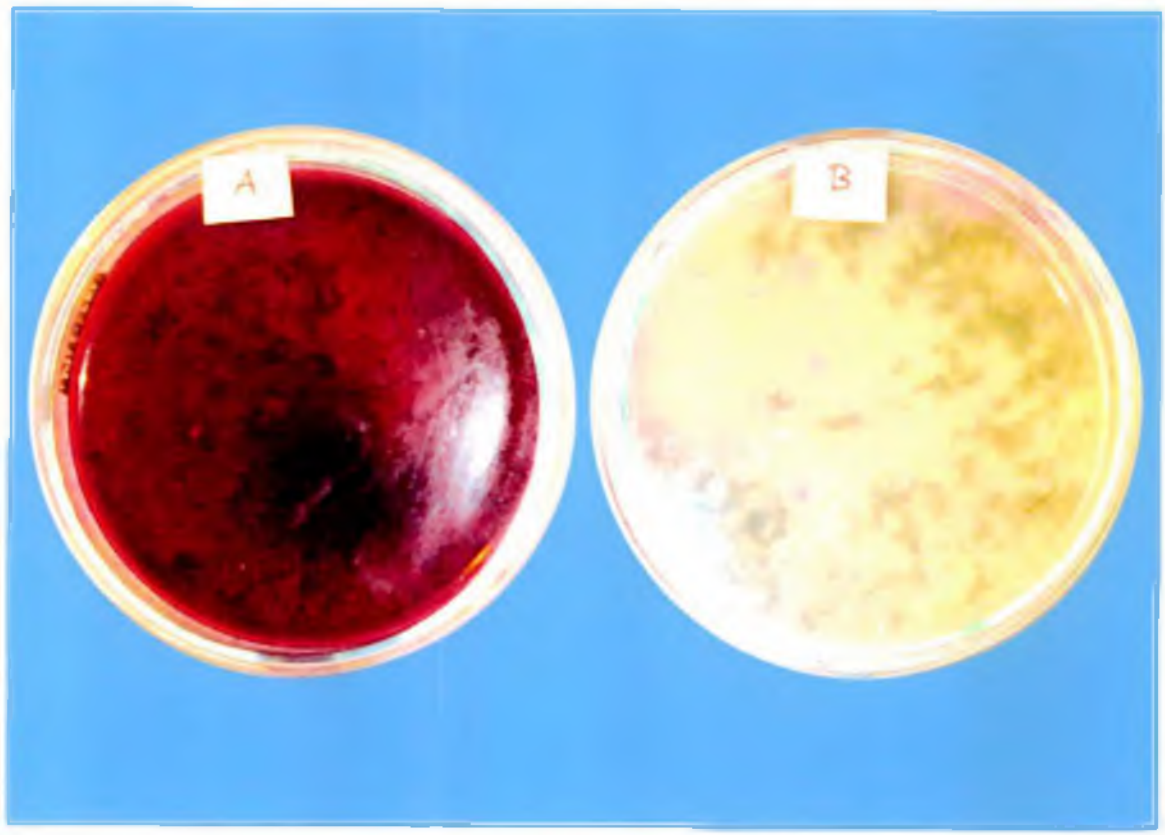

Fig-25: A. Dye pollutant B. Tannery pollutant.

Biosorption capacity of *N. crassa* biomass with mixed pollutants (Tannery pollutants and Dye pollutants)

Result of mixed pollutants (Tannery and Dye pollutants)

Neurospora crassa vigorous mutants and wild type Ema were selected. Biomass was produced and added with those pollutants and observed after 3, 6 & 9 hour.Absorbtion and Colour change of biomass was recorded.

Result for Tannery Pollutants

Table 17. Biosorption of Tannery Pollutant by wild type (Ema)

Sample Name	Amount of Sample	Observation after (hrs)	Quantity of Biomass		
			Before experiment (g)	After experiment (g)	Absorption (g)
Sterilized distilled water	10	3	1	1	0
Sterilized distilled water	10	6	1	1	0
Sterilized distilled water	10	9	1	1	0
Tannery pollutant	10	3	1	1.16	0.16
Tannery pollutant	10	6	1	1.29	0.29
Tannery pollutant	10	9	1	1.39	0.39

Note: No absorption incase of sterilized distilled water (control) & absorption in tannery pollutant.

Table 18. Biosorption of Tannery Pollutant by vigorous mutant.

Sample Name	Amount of Sample	Observation after (hrs)	Quantity of Biomass		
			Before experiment (g)	After experiment (g)	Absorption (g)
Sterilized distilled water	10	3	1	1	0
Sterilized distilled water	10	6	1	1	0
Sterilized distilled water	10	9	1	1	0
Tannery pollutant	10	3	1	1.2	0.2
Tannery pollutant	10	6	1	1.35	0.35
Tannery pollutant	10	9	1	1.47	0.47

Note: No absorption incase of sterilized distilled water (control) & absorption in tannery pollutant.

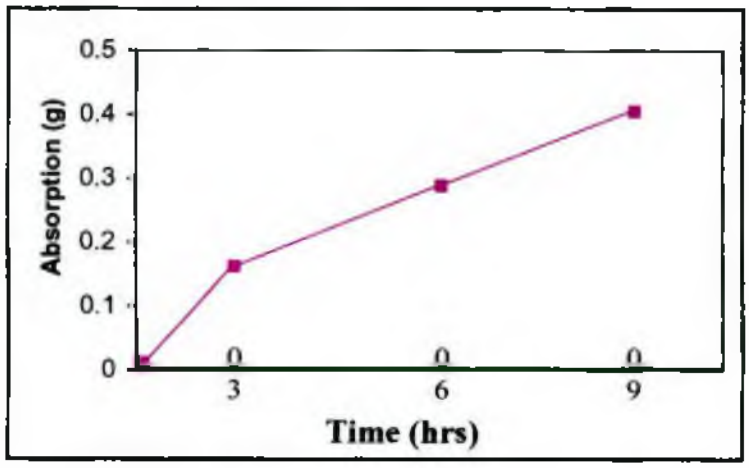

Fig 26. Biosorption of Tannery Pollutant by Ema.

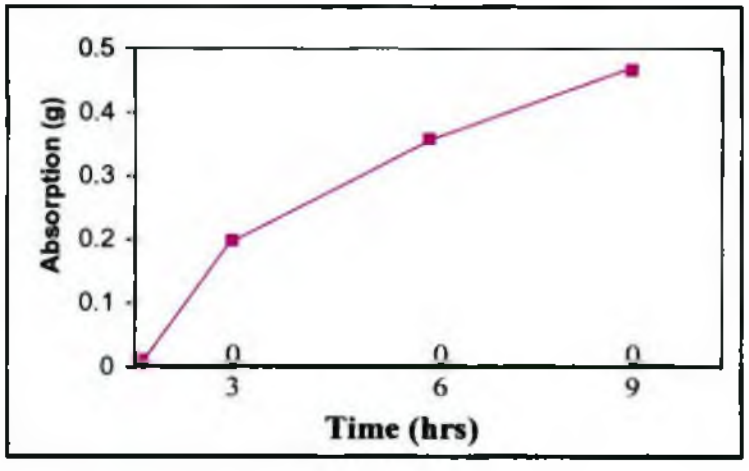

Fig 27. Biosorption of Tannery Pollutant by *vigorous* mutant.

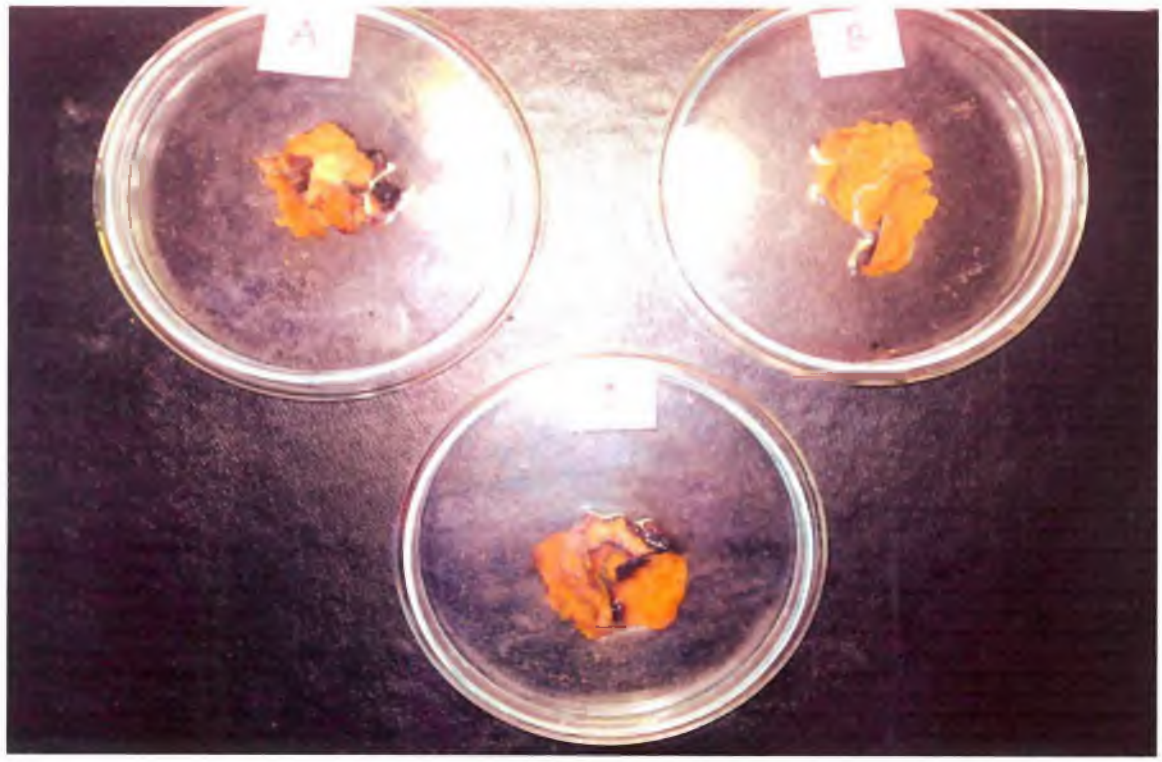

Fig-28: Biomass of Ema with control at a. 3, b. 6 & c. 9 hours.

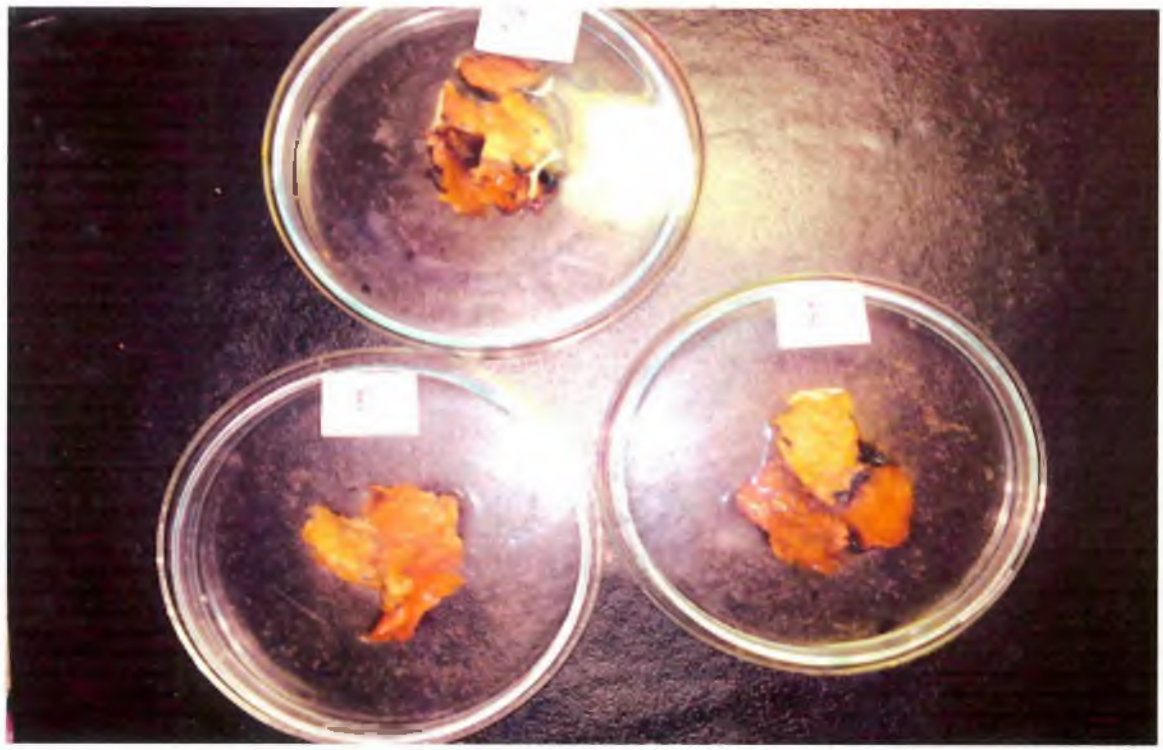

Fig-29: Biomass of vigorous mutant with control at a. 3, b. 6 & c. 9 hours.

Fig-30: Biomass of Ema with Tannery pollutants at a. 3, b. 6 & c. 9 hours.

Fig-31: Biomass of vigorous mutant with Tannery pollutants at a. 3, b. 6 & c. 9 hours.

Result for Dye Pollutants

Table 19. Biosorption of Dye Pollutant by wild type (Ema)

Sample Name	Amount of Sample	Observation after (hrs)	Quantity of Biomass		
			Before experiment (g)	After experiment (g)	Absorption (g)
Sterilized distilled water	10	3	1	1	0
Sterilized distilled water	10	6	1	1	0
Sterilized distilled water	10	9	1	1	0
Dye pollutant	10	3	1	1.04	0.04
Dye pollutant	10	6	1	1.07	0.07
Dye pollutant	10	9	1	1.09	0.09

Note: No absorption incase of sterilized distilled water (control) & absorption in dye pollutant.

Table 20. Biosorption of Dye Pollutant by vigorous mutant.

Sample Name	Amount of Sample	Observation after (hrs)	Quantity of Biomass		
			Before experiment (g)	After experiment (g)	Absorption (g)
Sterilized distilled water	10	3	1	1	0
Sterilized distilled water	10	6	1	1	0
Sterilized distilled water	10	9	1	1	0
Dye pollutant	10	3	1	1.05	0.05
Dye pollutant	10	6	1	1.09	0.09
Dye pollutant	10	9	1	1.12	0.12

Note: No absorption incase of sterilized distilled water (control) & absorption in dye pollutant.

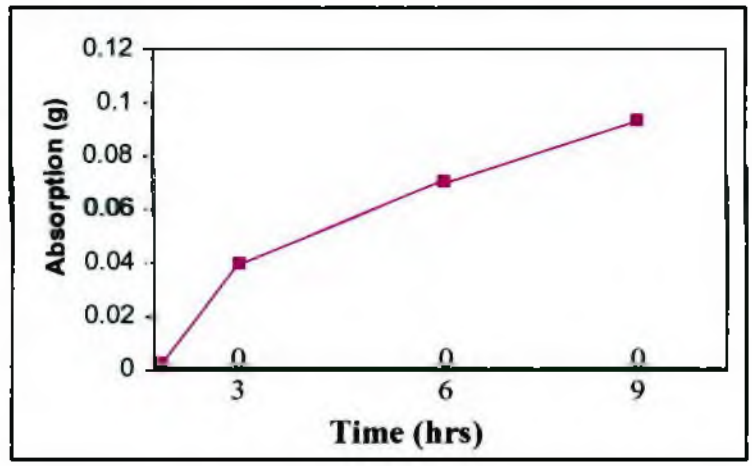

Fig 32. Biosorption of Dye Pollutant by Ema.

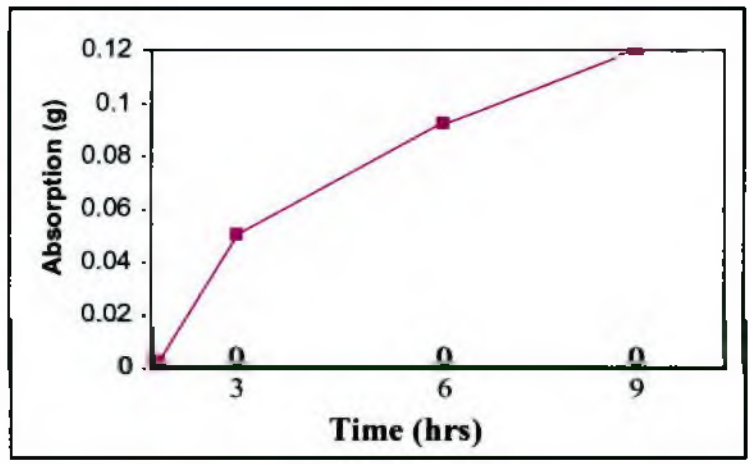

Fig 33. Biosorption of Dye Pollutant by vigorous mutant.

Fig-34: Biomass of Ema with Dye pollutant at a. 3, b. 6 & c. 9 hours.

Fig-35: Biomass of vigorous mutant with Dye pollutant at a. 3, b. 6 & c. 9 hours.

DISCUSSION

Neurospora crassa, a non-pathogenic filamentous fungus is widely used material for the study of genetics. It is not only used in the study of classical genetics but also used in the study of modern genetics as well as in the applied or practical field. Life cycle of the fungus is short and growth is vigorous. On the other hand by mutation many types of mutants can be found. Besides, the fungus and its mutants produce huge biomass and the fungus has biosorption capacity. By this capacity the fungus can adsorb the pollutants from nature and thus clean the environment.

For mutation purpose of *N. crassa*, extract of three plants were used which showed significant mutagenic effect and yield mutants. The extract of plants part (leaf or fruit) contains different chemicals which are called chemical mutagen. The plant parts were leaf extract of papaya (*Carica papaya* Linn) which contains alkaloids like nicotine, continine, myosmine etc. (Ghani-2003). Fruit extract of bitter gourd (*Momordica charantia* Linn) contain steroidal saponins, alkaloid momordicine, phenolic compounds etc. (Ghani-2003) and leaf extract of tea (*Camelia sinensis* Linn. O. Kuntze) contains alkaloid caffeine, theophylline, theobromine, xanthine etc (Ghani-2003). Before mutation, mutagenic effect of the extract of different plants part was studied as radial growth. The previous three plants showed significant mutagenic effect on *N. crassa*. For this reason, those 3 plants were selected for mutation.

Leaf extract of *Carica papaya* decreased radial growth of *N. crassa* mycelia. When the concentration of the extract increased, radial growth decreased gradually (Fig 2). This significant effect of leaf extract of *Carica papaya* was suitable to use this plant for mutation of *N. crassa*. After mutation of *N. crassa* conidia with leaf extract of *Carica papaya*, 5 types of mutants were found. These are *vigorous*, *cauliflower*, *fluffy*, *fluffy- band* and *conidial-band* (Fig. 4).

Fruit extract of *Mamordica charantia* also showed significant mutagenic effect. Radial growth was decreased with the increase of concentration of extract (Fig. 5).

After mutation of *N. crassa* with leaf extract of *Momordica charantia*, 4 types of mutants were found. These are *albino, dirty, ropy* and *vigorous* (Fig. 7). Leaf extract of *Camelia sinensis* also decreased redial growth of mycelia of *N. crassa* (Fig. 8). For this reason, this plant was also selected for mutation. After mutation of *N. crassa* with leaf extract of *Camelia sinensis*, 4 types of mutants were found. These are *conidial-band, fissure, fluffy* and *plug* (Fig. 10).

As physical mutagen, UV ray (254 nm) was used following filtration enrichment method of Catcheside (Fig. 14) after mutation of *N. crassa* with UV ray, 4 types of mutants were found. These are *vigorous, fluffy, conidial-band* and *cauliflower* (Fig. 15).

From these all mutation using physical and chemical mutagen, altogether 10 morphological mutants *(vigorous, cauliflower, fluffy, fluffy-band, conidial-band, albino, dirty, ropy, fissure* and *plug)* were found.

The mutants were studied in all possible ways. These are mycelial growth, linear growth and dry weight of mycelia.

Out of 10 mutants, *vigorous* showed highest mycelial growth and it is 2.5 times higher than the wild type (Fig. 16, 17 and 18)

In case of linear growth, *vigorous* showed highest growth. Besides *fluffy, fluffy-band, plug, conidial-band, cauliflower, ropy* etc. also showed good result.
In case of dry mycelial weight, *vigorous* showed highest weight. *Fluffy, Fluffy-band, albino* and *plug* also showed good result.

The pollutants were divided into two groups. These are single pollutants and mixed pollutants. The pollutants were As, Pb, Dye pollutants & Tannery pollutants.

The research work was accomplished both in the laboratory and outside. Some samples were collected from outside (mixed pollutants) and some were prepared in the laboratory (single pollutants). The single pollutants (As & Pb) were tested with *N. crassa* biomass at BCSIR, Dhaka and the mixed pollutants (Dye pollutants & Tannery pollutants) were tested with *N. crassa* biomass in the laboratory of the Department of Botany, Dhaka University. In both case, the *'biosorption'* capacity of the fungus was experimented.

Out of 10 mutants, as the *vigorous* mutant showed highest growth, this mutant was utilized for the study of biosorption capacity with single pollutants (As & Pb) at BCSIR laboratory.The growth of this mutant is higher than the fast growing mutant of the work Mozmader *et al.*(1997).

Single pollutants (As & Pb) with other chemicals were mixed with normal water separately at BCSIR laboratory. Then biomass was added with these pollutants to observe biosorption capacity of the mutant *vigorous*.

Mixed pollutants were collected from two spot. One is Jagannath Hall, Dhaka University, where the colour pollutants (Fig 21) of the laundry cloth are discharged by the washerman. These pollutants pollute the pond water (Fig. 22) of the hall. Another spot is situated near the Leather technology college, Hazaribag, where the Tannery pollutant (which contain many chemicals and other substances) are discharged (Fig. 23) these pollutants also cause great environmental pollution by mixing with normal water and producing bad smell.

The result may be analysed into two ways. These are qualitative analysis and quantitative analysis.

Qualitative analysis includes change of the colour of biomass and this is possible due to biosorption capacity of the fungus. If colour of biomass is changed after experiment, than the result is significant and if the colour of biomass is unchanged, than the result is insignificant.

In case of As & Pb, biomass of vigorous mutant was used for study of biosorption capacity (Fig 24). But in this case, the mutant showed insignificant biosorption capacity. Neither colour of biomass changed nor the amount of pollutant decreased. But Mondal *et al.* (2008) reported the removal of waste water arsenic by some bacteria.It implied that bacteria are more efficient for removal of arsenic than fungus.

In case of Dye & Tannery pollutants, biomass of *vigorous* and wild type (Ema) was used to study biosorption capacity. Biomass of these all was placed into separate petridish. The Dye &Tannery pollutants were also placed into each petridish before placing of biomass. Amount of each pollutant was 10 ml and weight of biomass was 1 gm in each case. . At first Tannery pollutant was placed into three separate Petridish for 3, 6 & 9 hours. Biomass of Ema was placed into those petridish. After 3, 6 & 9 hours biomass was taken off. The same process was followed for vigorous mutant.

It was observed after 3 hour that biomass of *vigorous* mutant and wild type *N. crassa* (Ema) absorbed the pollutant present in the sample. Colour of the biomass was also changed.Absorption of vigorous mutant is slightly higher than Ema (Table 17 & 18). At 6&9 hours, absorption was increased but rate was slightly decreased (Fig 26 & 27). It implied that much of the absorption take place at the beginning of time.

In case of Dye pollutants, the same procedure was followed like the Tannery pollutants. Biomass of *N. crassa* wild and *vigorous* mutant absorbed the pollutants (Table 19 & 20).In this case absorption was increased with the increase of time but rate was slightly decreased (Fig-30 & 31). Absorption of *vigorous* mutant is slightly higher than wild *N.crassa* (Ema).The colour of biomass was also changed.

More or less similar works were investigated incase of heavy metals (Matheical *et al.* 1987-*E radiata*, Galun-a.1983, Galun-b. 1987-*Penicillium*, Tsezos and Velosky-1982-*Rhizopus*, Kapoor *et al.* 1999-*Aspergillus*, Mullen *et al.* 1992-*Aspergillus*, Treen-Sears *et al.* 1984-*Rhizopus*, Zhang *et al.* 1998-*Rhizopus*. Huang *et al.*1990-*Saccharomyces*, Khovrychev. 1973-Candida) with various fungus.

This quantitative and qualitative study indicates the significant biosorption capacity of *N.crassa* wild type (Ema) and its *vigorous* mutant on Tannery and Dye pollutant.

These results implied that the fungus *N. crassa* and its *vigorous* mutant showed good qualitative and quantitative result which indicated significant biosorption capacity of the fungus and its *vigorous* mutant.

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