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Growth Inhibition and Mutagenesis of *Neurospora crassa* and the Same Organism by Leaf Extracts of *Andrographis paniculata* Burm. f.

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Abstract

Crude leaf extracts of *Andrographis paniculata* Burm. f. showed significant inhibition of growth and mutagenesis of *N. crassa.* Result showed that 1 ml extract can react with the test organism, *Neurospora crassa* very easily. During the period of 24 hours, it produced only 1.8 cm linear vegetative growth of the mycelia. Whereas 1 ml and 0.50 ml extracts produced less reactions (2.95 and 3.15 cm. respectively). The linear growth decreased with the increase of the concentrations of the extracts. Conidia of Ema (5297) of *N. crassa* were treated for 3-4 hours separately in 100% and 50% concentration of the extracts. One hundred percent extracts produced four types of mutants, namely- *dirty, ropy, albino,* and *buff* and 50% extracts produced three types of mutants, namely- *plug, ropy* (thin) and *conidial brand*.

Key words: *Neurospora crassa, Andrographis paniculata, Mutants, Dirty, Ropy, Albino, Buff, Plug, Ropy* (thin) and *Conidial brand.*

Introduction

Neurospora crassa is the most interesting material for genetic study. Genetic work with this fungus effectively began sixty six years ago. The experimental material *N. crassa* is a well- known pink bread mould. It is a filamentous fungus that belongs to the class-Ascomycetes.

Their extremely short life cycle and capacity to grow in minimal medium help the scientists of this branch to perform extensive work within a period of short time which is one of the most essential factors of genetical study (Watson, 1970).Beadle and Tatum (1941) first used the N. crassa in genetical and at the same time for biochemical study.

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N. crassa was originally selected for studying biochemical genetics because it is an organism in which both formal genetic analysis and investigation of nutritional mutants appeared possible (Beadle and Tatum, 1941). Plant extracts play an important role to check the growth of various fungi. Scientists are interested in evaluating the antifungal activities of plant extracts against plant pathogenic fungi (Ahmed and Sultana, 1984; Bashar and Rai, 1991; Anwar *et. al.,* 1994). Haque and Shamsi (1996) observed that leaf extracts of neem (*Azadirachta indica*) have antifungal properties and it decreased the radial growth of fungus but none of them studied the mutagenic effect of the plant extracts. A plan was also 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, heterocaryosis in comparison with the wild type Ema. Chemical mutagens have the ability to penetrate cells and to change the DNA. Presently scientists are interested to evaluate the mutagenic properties of chemicals on *N. crassa*. Kalomegh is a well-known medicinal plant. So the present study was undertaken to determine the mutagenic and growth inhibitory effect of leaf extracts of kalomegh (*Andrographis paniculata*).

Materials and Methods

N. crassa Ema (5297) was the experimental material. The wild type strain was received from Fungal Genetic Stock Centre, Department of Microbiology, University of Kansas Medical School, Kansas, U.S.A. Strains Ema (5297) were used. Vogel's minimal medium (VM) (Vogel, 1956) was used for maintaining the cultures. Solid VM was used for obtaining and measuring linear growth of conidia (Ryan *et. al.,* 1943). Different concentrations of aqueous extracts of leaves of *A. paniculata* were used in the experiments. The extraction procedure are given below:

Aqueous extract: Mature fresh green leaves of *A. paniculata* was washed with sterilized distilled water and then air dried and 100 g of clean leaves was ground with mortar and pestle. The paste was filtered to get extracts. The filtered extract was centrifuged for 5 minutes in 300 rpm at 25°C. The supernatant was used for this experiment.

Preparation of solution of different concentration of Kalomegh (*Andrographis paniculata*):

Experiments were made to find out the mutagenic effect of Kalomegh extracts on *N. crassa.* Leaf extracts of different density were prepared with the help of sterilized distilled water. Leaves of different weight were taken separately, washed 3-4 times with tap water and 3 times with distilled water. After air drying, the leaves were ground in an electric grinder with distilled water and without distilled water. The paste was filtered with a sterilized cotton cloth and finally filtered with filter paper to get clean solution.

Different extracts of known strengths were prepared using leaves and sterilized distilled water. Twenty g leaves were pasted without distilled water and it was as N_P. The next solution of 20 g leaves and 10 ml distilled water (N_1), 10 g leaves and 10 ml distilled water (N_2), 10 g leaves and 18 ml distilled water (N_3), 10 g leaves and 20 ml distilled water (N_4), and 10 ml distilled water and 4 g leaves (N_5) were prepared which were denoted as N_1, N_2, N_3, N_4 and N_5 (Table I). All extracts namely N_p, N_1, N_2, N_3, N_4 and N_5 were preserved separately in sterilized test tubes in refrigerator. Some of the extracts were also sterilized in autoclave.

Preparation of different extracts of Kalomegh leaves

Different concentrations of the leaf extracts of Kalomegh with sterilized distilled water were prepared.

Table I. Different concentrations of Kalomegh solution

Name of the solution	Amount of leaf	Amount of sterilized distilled water
N_P	20g	-
N_1	20g	10ml
N_2	10g	10ml
N_3	10g	18ml
N_4	10g	20ml
N_5	4 g	10ml

Effect of water extracts of Kalomegh on the growth of *Neurospora crassa*

For testing the effect of water extracts of Kalomegh on the growth on *N. crassa*, with different concentrations of it were prepared. Different solutions were taken separately on the petridish at the rate of 1 drop, 2 drops, 3 drops, 4 drops, 8 drops, 0.5 ml, 1ml, 1.5ml, 2ml, 3 ml and 4ml. Ten ml of molten SM medium was added in each Petri dish and rotated gently for uniform mixing of solution with the medium. When the medium became solid the centre of the Petri dish was marked and fresh culture of Ema was inoculated at that point with a sterilized needle. All the Petri dishes were kept in an incubator at $25^{o}C$ after 16, 24, 40 and 48 hours, the radial growth of Ema of each petridish was measured in cm.

Treating of Ema with extracts of Kalomegh leaves:

a) Obtaining fresh culture

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

b) Sterilization

All the media, essential elements and instruments were sterilized in the autoclave at $120^{o}C$ under 15 Ib pressure for 20 minutes. The inoculation chamber, needle, centrifuge

machine etc. were also sterilized with rectified spirit.

c) Centrifugation

Ten ml Kalomegh leaf extracts of the concentration N_p, N_1, N_2, N_5 were taken into 4 centrifuge tubes. One loop of conidia (about 10,000 conidia) of Ema was taken into each tube and were shaken for homogenous solution. The solution was centrifuged with the help of a centrifuge machine for 20 minutes in 300 rpm at 25°C.

d) Filtration

After centrifugation, the solution above conidia was poured out from the centrifuge tube. Ten ml of sterilized distilled water was added to the centrifuge tube and centrifuged for 3 minutes. Then the distilled water was poured out. The same procedure was repeated twice.

e) Preparation of suspension with treated conidia

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

f) Plating of treated conidia

The sterilized Petri dishes were marked as N_p, N_1, N_2, N_5 and 1 drop of the suspension of each was taken. Accordingly, 10 ml of molten SM medium was added to a Petri dish and were shaken gently to mix with the suspension and media. The plates were kept inside the incubator at 25°C 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 can be isolated only.

h) Classification

After 5 days, all the cultures were observed and classified by comparing their characters with wild type Ema (Table II). The conidial cultures with any morphological variations were subcultured several times in small tubes and checked carefully whether any permanent morphological change occurs.

Growth test of different morphological mutants on SM, VM (sucrose) and VM (glucose)

Growth tests were made in sterilized Petri dishes 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.

Table II. Characteristics of the mutants of *N. crassa* obtained by the induction with Kalomegh (all concentrations).

Name of the mutant	Characteristics of the mutants	Aqueous extracts of Kalomegh
Ropy	The mycelia look like beautiful ropes, conidia pinkish orange in colour. Growth is less than wild type.	100% extracts.
Albino	Less growth of mycelia, conidia are very scanty in number. Mycelia and conidia are completely colourless.	100% extracts.
Buff	Growth checked and mycelia lie on the surface of the media. Conidia buff in coloration	100% extracts.
Plug	The mycelial growth reach the plug of the tube, deep pink conidia are formed outside the tube, conidia around the plug.	50% extracts.
Ropy(Thin)	Mycelia form rope like structure and conidia pink.	50% extracts
Dirty	Small conidial lump scattered here and there in the tube.	100% extracts.
Conidial brand.	Dense conidial growths form a band shaped structure at the top.	50% extracts.

Then conidia from a particular mutant were put in one compartment, 23 compartments contained conidia from different mutants and one contained wild type (Ema) as control.

Study of mutagenic effect of extracts of Kalomegh (*Andrographis paniculata*) on *Neurospora crassa*

Mature fresh green leaves of Kalomegh were washed with sterilized distilled water and then air dried. Fifty gm. of clean leaves were ground with mortar and pestle. The paste was filtered through extracts. The filtered extract was centrifuged for 5 minutes. The supernatant was used for this experiment. Six sets of experiments were set taking 0.5 ml, 1 ml, 1.5 ml, 2 ml, 3 ml and 4 ml of extracts. Conidia of Ema (5297) of *Neurospora crassa* were treated for 3-4 hours separately in 100% and 50% concen-

tration of the extracts. It was centrifuged and the supernatant was discarded, treated conidia were washed twice with sterilized distilled water by pouring 1 ml sterilized distilled in each centrifuge tube. Final suspension was made with sterilized distilled water. Then, 1 drop, 2 drops, 3 drops, 4 drops and 8 drops of conidial suspension were taken in each of the 30 Petri dishes {Table-III (i) and (ii)} and observed radial growth of Ema on VM containing Kalomegh (Andrographis paniculata Burm.) extracts. Vogel's minimal medium (VM) was poured in each of the 30 Petri dishes. Plates were incubated for formation of conidial colonies for 3 days. Observation was made each day for appearance of the colony. The colonies were isolated in the test tubes containing Vogel's minimal medium (VM) and incubated the isolates for growth at 25°C. After 4 days, the isolates were examined and classified (Table II). Different concentrations of Kalomegh extracts were used to test the mutagenic effect on *N. crassa.* Fixed concentrations had appropriate mutagenic effect on *N. crassa.*

Results and Discussion

The list of chemical substances which shown mutagenic activities become longer with every passing year. The authors tested the mutagenic effect of leaf extract of Kalomegh (*Andrographis paniculata* Burm. f.) on *N. crassa.* The plant contains a resinous substance, Kalmegh, a bitter crystalline diterpene lactone, andrographolide, ditermene glucoside neoandrographolide, deoxyandrographolides, epigeninm ethers and oter flavoroids and phenols. Leaves contain β-sitosterol glucoside, andrographolide and panicolide and polyphenols, caffeic and cholorogenic acids and a mixture of dicaffeolquinic acid (Gani, 2003). To determine the efficacy of kalomegh on the radial growth of *N. crassa* different concentrations of leaf extracts (sterilized and non-sterilized) were used. It was evident from Table III (i, ii) that the radial growth of *N. crassa* was proportional to the concentrations of kalomegh extracts used. By increasing the concentration of leaf extracts of kalomegh radial growth of Ema was reduced in comparison to control. N_p concentration was found to be more effective than N_1, N_2, N_3 and N_4 concentrations. An extensive study was conducted on different concentrations so that *N. crassa* may tolerate little checked growth and also to know the amount necessary to kill the fungus *N. crassa*. From the Table III (i and ii) it was noted that 2 ml of concentrations decreased the radial growth extensively and the colony became very compressed and checked as compared to the control and 2 ml of concentration killed the fungus *N. crassa.* There

Table III (i). Effect of Kalomegh extracts on the radial growth of *N. crassa* Ema.

Concentration of the solution	Solution type	Treatment time (hr.)	Amount of solution taken in a Petri dish and growth obtained in cm											
			0 drop (control)	1 drop	2 drop	3 drop	4 drop	8 drop	0.5 ml	0.1 ml	1.5 ml	2 ml	3 ml	4 ml ml
10 g leaves + 20 ml distilled water (N_4)	Sterilized	16	1.90	1.90	1.80	1.70	1.60	1.40	3.40	3.20	2.10	1.90	1.20	1.10
		24	2.50	2.30	2.20	2.10	2.00	1.90	3.15	2.95	1.50	1.20	0.90	0.80
		40	over	4.10	4.00	3.90	3.80	3.70	3.60	3.20	3.00	2.60	2.00	1.60
		48	over	4.40	4.30	4.20	4.10	4.00	3.90	3.60	3.00	2.50	2.00	1.90
	Unsterilized	16	1.90	1.90	1.80	1.70	1.60	1.40	4.45	3.20	2.10	1.90	1.70	1.60
		24	2.50	2.30	2.20	2.10	2.00	1.90	3.15	2.95	1.50	1.20	0.80	0.70
		40	over	4.10	4.00	3.90	3.80	3.70	3.60	3.20	3.00	2.60	2.00	1.50
		48	over	over	4.40	4.30	4.20	4.10	4.00	3.90	3.60	3.00	2.50	1.90
10 g leaves + 18 ml distilled water (N_3)	Sterilized	16	1.90	1.80	1.60	1.50	1.50	1.35	4.10	4.00	3.80	2.80	1.70	1.60
		24	2.50	2.50	2.10	2.00	2.00	1.90	3.15	2.95	1.20	1.00	0.80	0.70
		40	over	4.20	4.10	4.00	3.90	3.80	3.70	3.30	3.10	2.80	2.20	1.90
		48	over	over	over	4.30	4.20	4.10	4.00	3.60	3.40	3.00	2.40	2.00
	Unsterilized	16	1.90	1.80	1.60	1.50	1.40	1.35	4.10	3.90	2.80	2.70	1.60	1.50
		24	2.50	2.50	2.30	2.10	2.00	1.90	3.15	2.95	1.20	1.00	0.80	0.70
		40	over	4.20	4.10	4.00	3.90	3.80	3.70	3.30	3.10	2.80	2.20	1.90
		48	over	over	over	4.30	4.20	4.10	4.00	3.60	3.40	3.00	2.40	2.00

Table III (i). Effect of kalomegh extracts on the radial growth of *N. crassa* Ema.

Concentration of the solution	Treat-ment time (hr.)	Amount of solution taken in a Petri dish and growth obtained in cm											
		0 drop (control)	1 drop	2 drops	3 drops	4 drops	8 drops	0.5 ml	0.1 ml	1.5 ml	2 ml	3 ml	4 ml
10 g leaves + 10 ml distilled water (N_2)	16	1.90	1.40	1.20	1.10	1.00	1.00	3.90	3.70	2.50	2.30	0	0
	24	2.50	2.40	2.35	2.30	2.20	1.90	3.15	2.95	1.10	1.00	0.40	0.35
	40	over	3.50	3.40	3.30	3.20	3.00	2.90	2.70	2.20	1.50	0.90	1.50
	48	over	4.00	3.90	3.80	3.60	3.30	3.20	3.00	2.50	1.50	1.20	0.60
10 g leaves + 10 ml distilled water (N_1)	16	1.90	1.00	0.90	0.80	0.70	0.60	3.30	3.10	2.70	2.30	1.60	1.20
	24	2.50	1.80	1.60	1.65	1.50	1.40	3.15	2.95	1.30	0.25	0.10	0
	40	over	3.80	3.40	3.30	3.20	3.00	2.80	2.60	0.70	0.50	0.35	0.20
	48	over	4.20	4.00	3.80	3.60	3.40	3.00	2.90	1.30	0.80	0.50	0.30
20g leaves (N_P)	16	4.90	4.70	4.20	3.50	3.30	3.20	3.40	3.10	2.60	2.10	1.80	1.40
	24	4.80	4.50	4.30	3.70	3.50	3.30	3.15	2.95	2.10	1.80	0.90	0.50
	40	over	over	over	4.20	4.10	3.90	3.80	3.50	2.40	2.30	2.10	1.80
	48	over	over	over	4.70	4.40	3.30	4.10	3.90	3.80	3.40	3.30	2.90

was no notable difference between sterilized and non-sterilized concentrations on the growth of *N. crassa* {Table III (i and ii)}. Crude extracts of the leaves of *A. paniculata* showed significant inhibition of growth and mutagenesis on *N. crassa.* Result showed that 1 ml extract reacted with the test organism of *N. crassa* effectively. During the period of 24 hours, it gave only 1.8 cm linear vegetative growth of the mycelia. Whereas 1ml and 0.50 ml extract produced less reactions (2.95 and 3.15 cm. respectively). The linear growth decreased with the increase of the concentration of the extracts.

Conidia of Ema (5297) of *N. crassa* were treated for 3-4 hours separately in 100% and 50% concentrations of the extracts. One hundred percent extracts produced four types of mutants, namely- *dirty, ropy, albino,* and *buff* and 50% extracts produced three types of mutants, namely *plug*, *ropy* (thin) and *conidial brand*. So, total mutants were of 7 types (Fig.1 and 2 and Table.II). The types and frequency of mutants obtained with the leaf extracts of *A. paniculata* were slightly different from that recorded earlier with leaf extract of *Azadirichta indica* (Keya, 1998) and bulb extract of *Allium sativum* (Yesmin, 1998).

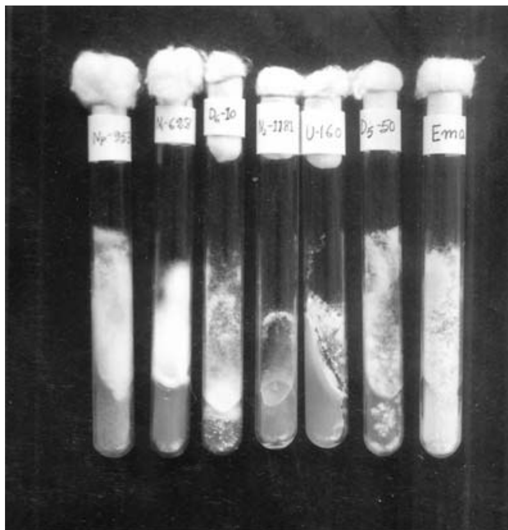

Fig 1: Growth pattern of different mutants of *N. crassa* with wild type Ema.

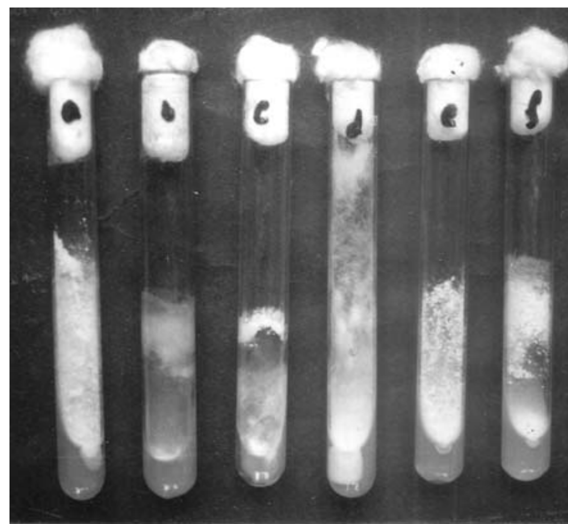

Fig 2: Growth pattern of different mutants of *N. crassa.*

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