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Microbial Antagonism and Induction of Mutation in Neurospora Crassa by Crude Leaves Extract of *Averrhoa carambola*.L.

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Abstract

Crude extract of leaves from *Averrhoa carambola* .L showed significant inhibition of growth and mutagenesis in *Neurospora crassa*. Result showed that 1 ml extract reacted with the test organism, *N. crassa very* much. During the period of 24 hours it gave only 2.4 cm linear vegetative growth of the mycelia. Whereas 0.5 ml and 0.25 ml extracts gave less reactions (1.75 cm and 2.25 cm respectively). The linear growth decreased with the increased of the concentration 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. 100% extracts produced 4 types mutants namely *ropy, albino, dirty and buff*. 50% extracts produced 4 types of mutants namely- *plug, vigorous, pigmented and conidial band.*

Key words: *Neurospora crassa, Averrhoa carambola*, mutants, *ropy, albino, dirty,buff, plug, vigorous, pigmented* and *conidial band.*

Introduction

Beadle and Tatum (1941) first used the *N. crassa* in genetically and at the same time for biochemical study. *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.

Mutagens are physical or chemical agents such as radiation, heat or alkalizing or determinations agents, which raise the frequency of mutation, greatly above the spontaneous background level. Chemical mutagens have the ability to penetrate cells and to alter the DNA within cell (Fishbein *et. al*., 1970), Chemicals that have mutagenic properties include peroxides, formaldehyde (Gardner, 1972), Permanganate, urethane, nitrous acid, Ethyl Methyl Sulphonate (EMS), Mustard gas (Altenberg, 1957), Chloramphenicol etc.

In 1939, Steinberg first reported the production of mutation by chemical treatment of cells. Study of Kolmarks and Westergaard (1949) have shown that Bromoethyl methane sulphonate when used on *N. crassa causes* mutation. Admirable accounts both of genetical and much of biochemical work is given by Cateheside (1949) and Horowitz (1973) . Perhaps Westergaard has made the most extensive and systemic surveys of the action of biochemical mutagens on fungi on *N. crassa* (Fincham and Day, 1965).

Mutation is the ultimate source of all genetic variation and it provides the raw material for evolution, Mutation provide decisive evidence that DNA is the genetic material. Artificial induction of mutation is one of the criteria to study the organization and mode of action of genes. Now a days a good number of physical and chemical mutagens are used by the geneticists for induction of mutation. Chemical mutagens have the ability to penetrate cells and to alter the DNA. Presently scientist are interested to evaluate the mutagenic properties of chemicals on *Neurospora crassa*. The experimental material *N. crassa* is a well-known pink bread mold. It is a filamentous fungus that belongs to the class-Ascomycetes. 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; Miah *et. al.*, 1990; Bashar and Rai, 1991; Anwar *et. al.*, 1994 .). Haque and Shamsi (1996) observed that leaf extracts of neem *(Azadirachta indica)* has antifungal properties and it decreased the radial growth of fungus but none of them studied the mutagenic effect of the plant extracts. The most technically advantageous method of studying the organization and mode of action of gene or the genie inheritance in any organism is achieved by inducing artificial mutation in the organism at different loci. Without mutation the loci could never be identified; nor their functions studied. The present study was undertaken to find the microbial antagonism and mutagenic effect of leaf extracts of *A. carambola* on *N. crassa* & thereby to produce mutation in *N. crassa. A. carambola*. L is a well-known medicinal plant (Family-Averrhoaceae).

Materials and Methods

*N. crass*a 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 the maintenance of cultures. Solid VM was used for obtaining and measuring linear growth of conidia (Ryan *et. al.*, 1943). Different concentration of aqueous extracts of leaves of *A. carambola* L. were used in the experiments, The extraction procedure are given below:

Aqueous extract: Mature fresh green leaves of *A. carambola* L. was washed with sterilized distilled water and then air dried.100 g of clean leaves was grinned with mortar and paste. The paste was filtered through extracts. The filtered extract was centrifuged for 5 minutes in 300 rpm at 25^{O} C. The supernatant was used for this experiment.

Conidia of Ema (5297) of *N. crassa* were treated for 3-4 hours separately in 100% and 50% concentration 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. One drop (about 10,000 µl inocula) of conidial suspension was taken in each of the 40 Petri dishes. Molten Sorbose minimal medium (SM) was poured in each to the 40 Petri dishes for mycelial linear growth. Plates were incubated at 25^{O} C for formation of conidial colonies for 3 days. Observation was taken each day for appearance of colony. The colonies were isolated in the test tubes containing Vogel's minimal medium (VM). Incubated the isolates for growth at 25^{O}C. After 4 days the isolates were examined and classified.

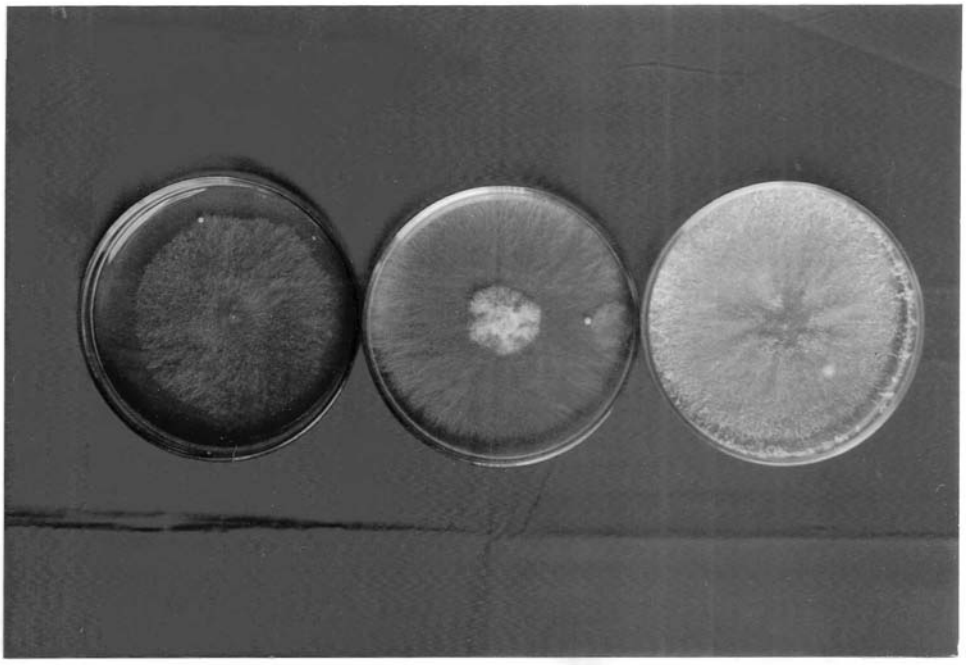

Fig. 1. Comparative studies of linear growth in different concentrations (0.25 ml, 0.50 ml and 1 ml concentrations respectively)

Results and Discussion

Crude extracts of leaves from *A. carambola* .L. showed significant inhibition of growth and mutagenesis *N. crassa*. Result showed that 1 ml extract reacted with the test organism, *N. crassa* very much. During the period of 24 hours it gave only 2.4 cm linear vegetative growth of the mycelia. Whereas 0.5 ml and 0.25 ml extracts gave less reactions (1.75 cm and 2.25 cm respectively). (Fig.1) The linear growth decreased with the increase of the concentration of the extracts.

Crude extracts of leaves from *A. carambola* L. Showed mutagenesis with the test organism, *N. crassa*. 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 four types of mutants, namely- *plug, vigorous, pigmented* and *conidial band*. So total mutants 8 types (Fig. 2 and Table.I).

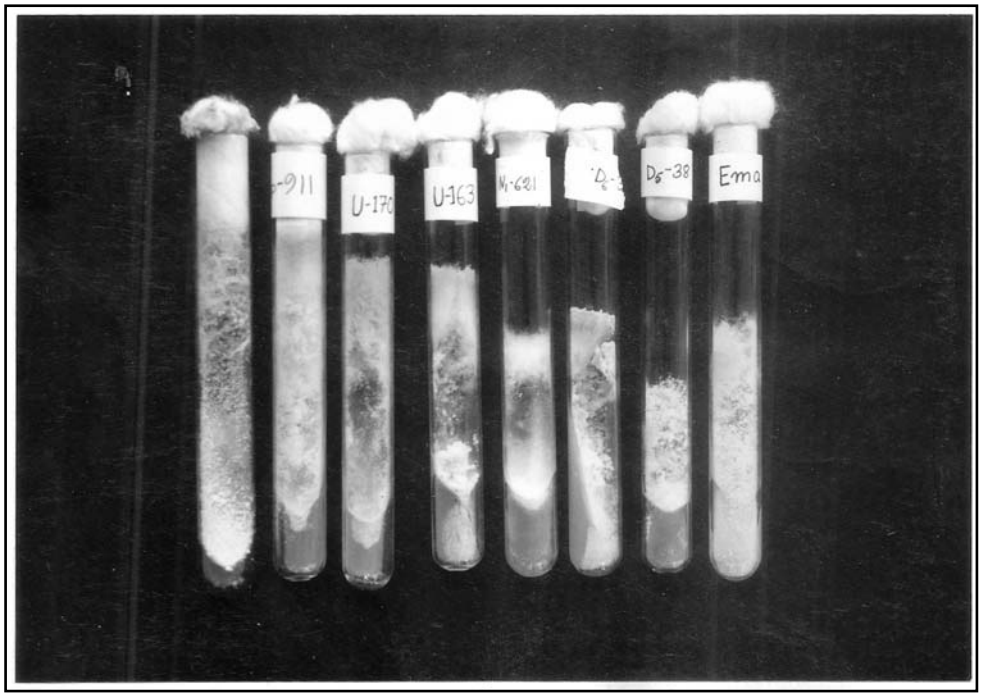

Fig. 2. *plug* (no labeling test tube), *vigorous* (U-911), *dirty* (U-170), *buff* (U-163), *albino* (N1-621), *ropy* (D6-3), *conidial band* (D5-38) and Ema mutants

Table I. Classification of the Neurospora crassa mutants

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.
dirty	Small conidial lump scattered here and there in the tube.	100% extracts.
buff	Growth checked and 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 formed outside the tube, conidia around the plug.	50% extracts.
vigorous (mycelial)	Densed mycelial growth, growth reach the plug, mycelia cottony.	50% extracts
pigmented	Mycelia rope like, deep orange colored pigmentation	50% extracts.
conidial brand.	Dense conidial growth form a band shaped structure at the top.	50% extracts.

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