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Inhibition of Growth of *Neurospora Crassa* and the Same Organism by Rhizome Extracts of *Zingiber Officinale*

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Abstract

It was observed that rhizome extract of *Zingiber officinale* A. juss possesses antifungal properties and it reduced the radial growth of *Neurospora crassa* Ema. After treating conidia of Ema with rhizome extract of *Z. officinale*, 10 groups of mutants were found i.e. *albino, fluffy, fluffy-band, yellow-fluffy, crescent, checked, conidial band, ropy, plug* and *vigorous.*

Key words: *Neurospora crassa, Zingiber officinale, Mutants, Albino, fluffy, Fluffy-band, Yellow-fluffy, Crescent, Checked, Conidial band, Ropy, Plug* and *Vigorous.*

Introduction

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 scientists are interested to evaluate the mutagenic properties of chemicals on *N. 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; 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.

Ginger is a well-known medicinal plant. The present study was undertaken to find the mutagenic and growth inhibitory effect of rhizome extracts of ginger (*Zingiber officinale* A. *juss*).

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. Strain Ema (5297) was used. Vogel's minimal medium (VM) (Vogel, 1956) was used for the maintenance of culture. Solid VM was used for obtaining and measuring linear growth of conidia (Ryan *et. al.,* 1943). Different concentrations of aqueous extract of Rhizome of ginger were used in the experiments, The extraction procedure are given below:

Aqueous extract

Rhizome of ginger was washed with sterilized distilled water and then air dried. Fifty g of clean rhizome were ground with mortar and paste. The paste was filtered. The filtered extract was centrifuged for 5 minutes. The supernatant was used for this experiment. For testing the effect different solutions -sterilized (Z_0, Z_1, Z_2, Z_3, Z_4) and non-sterilized (Z_5) were taken separately on the sterilized petridish at the rate of 1 drop, 2 drops, 4 drops, 8 drops, 1 ml, 1.5 ml, and 2 ml. 10 ml of molten VM was added in each petridish. When medium became solid Ema was inoculated at the centre of the medium. Plates were incubated at 25^{O}C and the radial growth was measured after 16, 24, 40 and 48 hours. Aqueous rhizome extracts of Ginger restricted the growth of *N. crassa.* But it was notable that there was no difference between sterilized and unsterilized solution on the growth of *N. crassa.* For that, further observation were taken with only unsterilized solution. From these studies data, induction of mutation have observed by the treated of conidia in different concentrations of media and found different types of mutants. It is clearly showed that here mutation should be occur. The results suggest that unique mutation can be generated by concentrations of media and that such mutants are a consequence of the dominance of a particular chemical class or classes within the media.

Results and Discussion

Spontaneous mutation in *N. crassa* is very rare and infrequent in nature. The author checked if there was any spontaneous mutation in *N. crassa*. In this investigation conidia from fresh culture of Ema (5297) were spread on SM plates. Distinctly grown single conidial colonies were isolated individually in VM tubes. After 4-5 days when sufficient conidia were formed, individual culture was

Table I. Effect of ginger extract on the radial growth of *N. crassa* Ema

Concentration of the solution	Solution type	Treating time (hr.)	Amount of solution taken in a petridish and growth obtained in cm.							
			0 Drop (concentration)	1 Drop	2 Drops	4 Drops	8 Drops	1 ml	1.5 ml	2 ml
	Sterilized	16	2.00	2.00	1.90	1.70	1.50	1.30	1.20	0.80
		24	2.80	2.30	2.10	1.90	1.60	1.40	1.30	0.60
Z_5		40	over	2.70	2.40	2.10	1.90	1.80	1.70	1.10
		48	over	2.90	2.50	2.20	2.10	2.00	1.90	1.20
		16	2.00	2.00	1.90	1.70	1.50	1.30	1.20	0.80
	Non sterilized	24	2.80	2.30	2.10	1.90	1.60	1.40	1.30	0.60
		40	over	2.70	2.40	2.10	1.90	1.40	1.70	1.10
		48	over	3.00	2.60	2.30	2.20	1.80	2.00	1.30

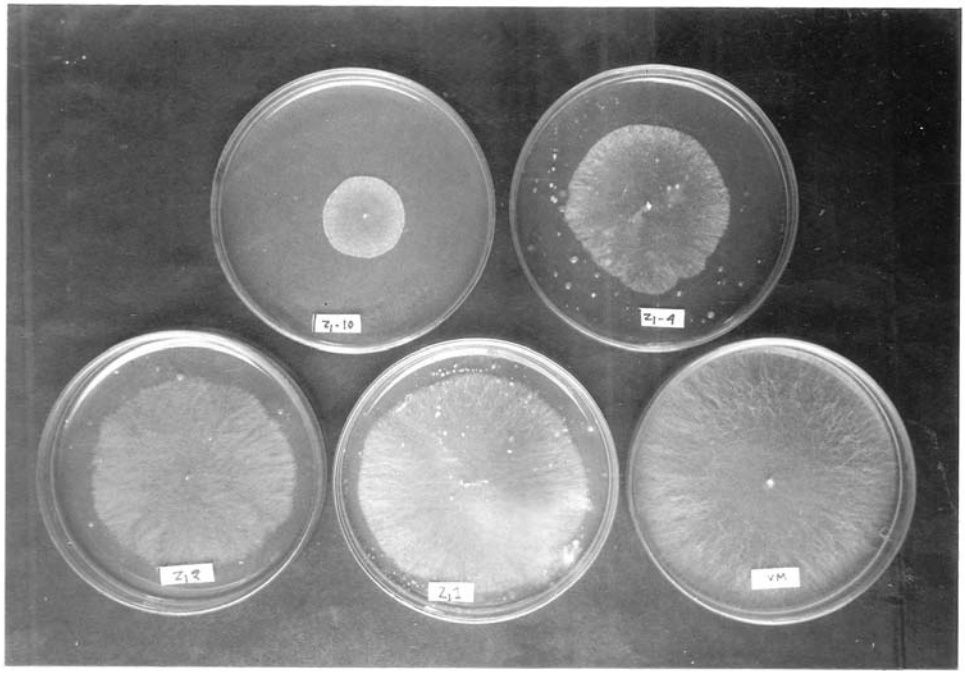

Fig. 1. Comparative radial growth of Ema on VM containing Ginger rhizome extracts of Z_o concentration

analyzed. None of the isolates were found to show morphological variations and all of them resembled the parental stock. It showed that there was no spontaneous mutation in *N. crassa* in laboratory stock which the author used for the induction of mutation.

To test the effectiveness of ginger on the radial growth of *N. crassa* different concentrations of ginger extracts (sterilized and non-sterilized) were used (Z_0, Z_1, Z_2, Z_3, Z_4 and Z_5). It was evident from Table I and Fig. 1 that the radial growth of *N. crassa* was proportional to the concentrations of ginger used. By increasing the concentration of rhizome extracts of ginger (*Z. officinale* A. *juss.*) radial growth of Ema was reduced in comparison to control. Z_0 concentration was found more effective that (Z_0, Z_1, Z_2, Z_3, Z_4 and Z_5)., concentrations. It was noted that 1 drop, 2 drops, 4drops, 8 drops, 1 ml and 2 ml of Z_5, Z_4, Z_3, Z_2, and Z_1, concentrations decreased the radial growth extensively and colony became very compressed and checked as compared to the control. 2 ml of Z_1 concentration killed the fungus *N. crassa.* There was no notable difference between-sterilized and non-sterilized concentrations on the growth of *N. crassa*. An experiment on the mutagenicity of ginger was performed Z_0 concentration in non-sterilized condition was chosen for treating conidia of Ema for

Table II. Characteristics of the mutants of *N. crassa* obtained by the induction with ginger (Z_0 concentation)

Name of the group	Characteristics of the mutants	No. of mutant	Name of the mutant
A	Colourless mycelia and conidia	3	*albino*
B	Profuse mycelial growth, no conidia	10	*fluffy*
C	Densed colourless mycelial band form at the tip	12	*fluffy band*
D	Yellow mycelial growth, no conidia.	8	*yellow-fluffy*
E	Conidial growth form a nice crescent at the tip of the growth	10	*crescent*
F	Growth very much less than wild	6	*checked*
G	Conidia formed a band at the tip of the growth	7	*conidial -band*
H	Mycelia rope like	7	*ropy*
I	Conidia formed outside tube over the plug	2	*plug*
J	Huge conidial growth touches the plug	10	*vigorous*

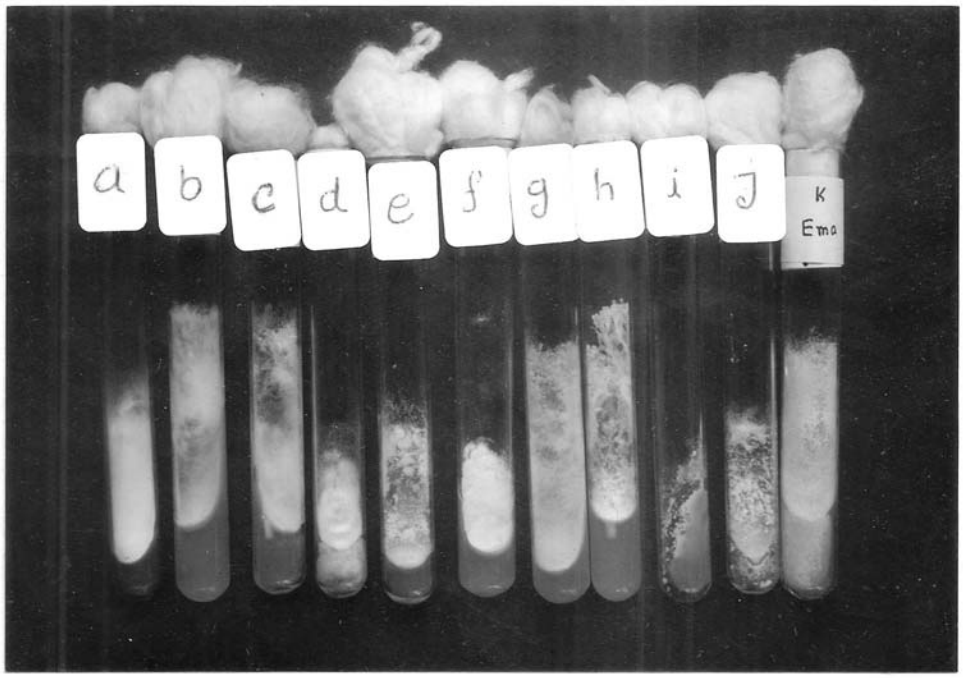

Fig. 2. Growth pattern of different mutants of *N. crassa*

induction of mutation. 10 groups of mutants were obtained Table II and Fig. 2 (*i.e. al, fl, fl-band, yfl, cr, ch, con-band, ro, pl, vg*).The types and frequency of mutants obtained with rhizome extracts of *Z. officinale* A. juss. were slightly different from that found earlier with leaf extract of *Azadirichta indica* (Keya, 1998) and bulb extract of *Allium sativum* (Yesmin, 1998).

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