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Screening of herbicides for posssible mutagenicity in the cyanobacterium *Nostoc linckia* and isolation of antibiotic resistant strains

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ABSTRACT

Herbicides Propachlor, basalin, propanil atrazine, monuron and Saturn belonging to different chemical groups were tested for their genetic effect on the N_2 – fixing cyanobacterium *N. linckia* for Kanamycin resistant (Knm^R) and their mutagenic potentials were evaluated in comparison to MNNG, a known mutagen to cyanobacteria, over spontaneous frequency. Propachlor, basalin and atrazine like MNNG were found to be more potent mutagen to induce forward mutation (resistant mutant) for Kanamycin (Knm^R) with more frequency than MNNG while propanil, Monuron and saturn were not able to increase the frequency above spontaneous level.

Key words: Herbicides, Antibiotics, Mutagen, Genetic effect, MNNG, *Nostoc linckia,* Kanamycin.

INTRODUCTION

The widespread and extensive use of herbicides in agriculture is to suppress weed growth and to increase the crop yield (Chattopadhyay, 1987). These chemicals have significant effect to agriculturally important N_2 – fixing cyanobacteria (Zargar and Dar,. 1990; Vaishampayan *et al* 2000).Such agro- chemicals causes much concern about possible genetic hazards to living system, if their residues are incorporated into food chain at various points for varying periods. Several of these compounds are not only mutagenic but are also carcinogenic to the living cells. (Eisenbrand *et al,* 1974; Seiler, 1978; Ames, 1979; Sugimura *et al*, 1981) and a large member of pesticides (including herbicides, insecticides, fungicides, nematocides etc.) have been found to be mutagenic in prokaryotic and eukaryotic plant and animal cells. (Anderson *et al,* 1972; Shirasu *et al,* 1976; Nagao *et al,* 1981). The cyanobacteria which play an important role in nitrogen economy of rice agriculture (Singh, 1961; Venkataraman, 1972; Singh and Pandey, 2007, a,b) invariably present at the time of herbicide application in the field have received little attention from biologist as they are unusual indicator organisms for this purpose (Singh *et al* , 1979; Pandey, 1999; Vaishampayan *et al,* 2000). There fore in view of their significant role in agriculture the present study was undertaken to evaluate the mutagenic effect of the commonly used herbicides for resistant of antibiotic Kanamycin (Knm^R) in N_2 – fixing cyanobacterium *Nostoc linckia* and their frequencies were compared over spontaneous and MNNG induced frequencies.

The clonal and axenic culture of heterocystous N_2 – fixing cyanobacterium *N. linckia* was routinely grown in modified Chu- 10 medium (Safferman and Morris, 1964) at the expens of elementary nitrogen. The temperature of incubation in growth controlled chamber was 28 **+** 2ÚC for the maintenance of the cyanobacterial culture and growth experiment. Growth controlled chamber was illuminated with cool day light fluorescent tubes (intensity approximately 2200 lux) for 14 hours photoperiod and 10 hours nyctoperiod.

Herbicides

Herbicides used were of commercial grade and their chemical designation, type and source are given in Table -1. The mutagenic effect of the herbicide was compared with MNNG, a known mutagen for cyanobacteria (Asato and Folsome, 1969; Kumar, 1975; Herdman, 1982).

Mutagenic Experiment

Axenic and homogeneous population of cyanobacterium *N. linckia* were treated with selected herbicides and their mutagenic efficiency was compared with spontaneous and MNNG induced frequencies . Dose and treatment duration is described in Table-2 . Untreated population served for spontaneous mutation. Samples were washed thoroughly by repeated centrifugation and inoculated into 10ml N_2–free medium and allowed to grow and segregate for 3 days. Treated and control cultures were centrifuged and harvested samples were plated on agar plates containing 10 µg/ml Kanamycin, since the parent organism was unable to grow with 5 µg/ml Kanamycin. Suspension of *N. linckia* diluted 1000 fold were inoculated on to basal agar medium without Kanamycin to determine the number of viable colony forming unit.

Plates were incubated in growth chamber to count viable mutant colonies. The stability of the Kanamycin resistant phenotype was tested by growing in Kanamycin free N_2–medium for 10 successive subculture and then into Kanamycin (10 µg/ml) supplemented N_2–medium. The results were statistically analysed by employing t-test to test the level of significance.

RESULTS AND DISCUSSION

Genetic toxicity of herbicide Propachlor, Basalin, Propanil,atrazine, monuron and Saturn were evaluated for induction of mutation in the cyanobacterium *Nostoc linckia* for its resistance to 10 µg/ml Kanamycin and compared with MNNG (N-methyl-N'- nitro-N-nitrosoguanidine.) (Table-2). The most potent mutagen known so for (Singh and Vaishampayan, 1978; Vaishampayan, 1982), MNNG is known to cause mutation in the replicating DNA chains of microorganisms (Drake and Blatz, 1976). *N. linckia* grew well in N_2–medium, however it failed to grow in N_2–medium containing 5 µg/ml Kanamycin. The frequency of spontaneous mutation to Kanamycin (Knm^R, 10 µg/ml^{-1}) resistance was $2.20 \times 10^{-6} + 5.3 \times 10^{-8}$ while MNNG induced frequency was $5.25 \times 10^{-5} + 6.5 \times 10^{-7}$. The mutational yield obtained by these herbicides is also comparable to MNNG-induced mutation for the same marker. The observed action of propachlor, basalin and Atrazine seems analogous to that of MNNG and helps their placing in the category of powerful mutagens for cyanobacteria. An increase in mutation frequency was noticed over spontaneous and NTG-induced frequencies with propachlor ($6.45 \times 10^{-5} + 3.1 \times 10^{-6}$),

Table - 1: Herbicides selected for mutagenic test in the cyanobacterium *N. linckia*

S. No.	Common Name	Chemical Designation	Solvent	Source
1.	Propachlor (Remrod)	2-chloro-N-isopropylac-etanilide	Water	Monsato Chemicals (Pvt) Missouri (USA)
2.	Basalin (Fluchloralin)	N-protyl-N-(2-hloroethyl)-2,6-dinitro-n-rifluromethyl -aniline	Water	BASF Aktien gesettschagh West Germany
3.	Propanil (Stam-f-34)	3,4-dichlorotropiominalide	Water	Bharat Pulv. Mills. Mumbai (India)
4.	Atrazine (Zeatin)	2-chloro-4-ethylamino-6-iso-propylamino-s-triazine	Ethanol	Bharat Pulv. Mills. Mumbai (India)
5.	Monuron	3-(4-chlorophenyl)-1, 1-dimethyl Urea	Water ethanol	Bharat Pulv. Mills. Mumbai (India)
6.	Saturn	5-(4-chlorobenzyl)-N, N-diethylthiocarbamate	Water	Kumai, Chemical Industries Co. Ltd. Tokyo (Japan)

Table – 2: Spontaneous and induced mutation frequencies (+ SE) in *N. linckia* for the resistance to 10 ìg/ml antibiotic Kanamycin as a result of treatment with various groups of herbicide and MNNG (treatments have been used at a 50 % survival dose).

Chemical for treatment (Herbicide)	Dose μg ml $^{-1}$	Treatment duration (min)	Mutation frequency (Kanamycin 10 μg ml $^{-1}$)
Propachlor	100	30	$6.45\times10^{-5} \pm 3.1\times10^{-6}$
Basalin (Fluchloralin)	100	30	$5.78\times10^{-5} \pm 3.0\times10^{-7}$
Propanil(Stam f-34)	50	25	$2.5\times10^{-6} \pm 5.3\times10^{-8}$
Atrazine(Zeatin)	100	40	$5.9\times10^{-5} \pm 1.85\times10^{-7}$
Monuron	100	40	$2.31\times10^{-6} \pm 4.35\times10^{-8}$
Saturn	200	10	$2.40\times10^{-6} \pm 5.65\times10^{-8}$
Sqontaneous	200	10	$2.20\times10^{-6} \pm 5.3\times10^{-8}$
MNNG	100	25	$5.25\times10^{-5} \pm 6.5\times10^{-7}$

Basalin($5.78\times10^{-5} + 3.0\times10^{-7}$) and atrazine ($5.9\times10^{-5} + 1.85\times10^{-7}$) while the mutant frequency of propanil, monuron and Saturn remained almost the same as for spontaneous mutation (Table-2). The observations were found significant (P<0.01). One of the Knm^R resistant clone of *N. linckia* was tested for the resistance to Kanamycin (10μg ml $^{-1}$) after 10 successive subcultures in N_2–medium only. It was found to retain the Kanamycin phenotype (forward mutation) suggesting for a stable mutation origin in the cyanobacterium *N. linckia .*

The observed growth dependent biological action of tested herbicide in *N. linckia* seems analogous to MNNG which is known to cause mutation in the replicating DNA in microorganisms under growth condition (Drake and Blatz, 1976) and may definitely effective mutagen in cyanobacteria for drug resistance. Spontaneous resistance for antibiotics in prokaryotes is well known (Hashimoto, 1960; Kumar, 1964). The resistance have been shown to be both nuclear or plasmid born trait and controlled by gene (s). The tested herbicides have alkyl/amide group like MNNG. There fore possibility to mutation might be like the mechanism known for alkylating agent as reported by Drake (1969) and Singer (1975). In light of the above observations it is concluded that such chemicals in agriculture should be used carefully to avoid genetic hazards, since many mutagens are reportedly carcinogenic to mammalian system (Ames, 1979).

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