



ANTIMUTAGENIC ACTIVITY OF GALLIC ACID ON THE GROWTH OF MUTAGENIZED *RHIZOBIUM* *MELILOTI* CULTURES

Manoj Kumar Rawat¹, P.C. Bhati², Rajendra Prasad³

^{1,3}Department of Botany, Government College Bundi-323001, Rajasthan (India)

²Department of Zoology, Government College Bundi-323001, Rajasthan (India)

ABSTRACT

Growth percentage of the sodium azide (NaN_3 ; pH 6.0) treated cultures of *Rhizobium meliloti* was used to assess the concentrations response of this mutagen (NaN_3). A gradual decrease in growth percentage was recorded with increasing concentrations of NaN_3 . However, antimutagenic treatment with gallic acid showed increase in values of growth percent in mutagenized *R. meliloti* cultures.

Key words: Antimutagenic, gallic acid, mutagenized, *Rhizobium meliloti*, sodium azide.

I. INTRODUCTION

The alarm is triggered by dangerous chemicals causing mutations and cancer which are detected in insecticides, food, fodder, atmosphere and drinking water. Toxicological studies have undergone a significant evolution during the past decades, with much greater emphasis being placed on chronic toxicity, carcinogenicity, teratogenicity and mutagenicity [1].

Further, widespread fear of cancer has generated awareness about scope of antimutagens and their antimutagenicity. A search for antimutagens and anticarcinogens are quite necessary to neutralize the harmful effects of a large number of chemicals present in our environment which are carcinogenic, mutagenic or teratogenic. A considerable interest has been generated to screen the natural substances or agents which are capable of neutralizing the effects of known mutagens and carcinogens[2]. Against this background, in the present study antimutagenic activity of gallic acid on the growth of mutagenized *R. meliloti* cultures using growth percent as the criterion has been assessed.

II. MATERIALS AND METHODS

R. meliloti obtained from mother variety of *Trigo nella foenum-graecum* was maintained on YEMA or CR YEMA media. For azide treatment, 0.1 ml exponential phase (24h old) cell suspension of rhizobial cultures were transferred to freshly prepared and filtered sterilized solutions of NaN_3 ($4 \times 10^{-5}\text{M}$; $9 \times 10^{-5}\text{M}$; $1 \times 10^{-4}\text{M}$) in 100 ml conical flasks. Flasks were kept for 24 hour at $28 \pm 1^\circ\text{C}$ temperature on a rotary



shaker. A set of untreated (normal) rhizobial cultures was run as control. One set of azide mutagenized rhizobial cultures was post treated with freshly prepared and filtered sterilized aqueous solutions of gallic acid in 100 ml conical flasks. The flasks were kept for 24 hour at $28\pm 1^{\circ}\text{C}$ temperature on a rotary shaker. Untreated, azide alone and azide in combination with gallic acid post treated cultures were serially diluted, plated and incubated to determine the influence of treatment on growth percentage.

III. RESULTS AND DISCUSSION

Growth percentage of the rhizobial cultures treated with mutagen alone and in combinations with gallic acid was used to assess the concentration response of these agents (mutagen and antimutagen). The recorded observations reveal that growth percentage in control was 100. NaN_3 treatments were found to be quite effective in reducing growth percentage. A gradual decrease in growth percentage (60.00; 40.00; 21.20) was observed with increasing concentrations of NaN_3 ($4\times 10^{-5}\text{M}$; $9\times 10^{-5}\text{M}$; $1\times 10^{-4}\text{M}$), respectively. $4\times 10^{-5}\text{M}$ concentration of NaN_3 has been used for post treatment with antimutagen (gallic acid), because the value of growth percentage was approximately 50. Higher concentrations of NaN_3 , i.e., $9\times 10^{-5}\text{M}$ and $1\times 10^{-4}\text{M}$ exerted significant negative effects on growth percent over control which was recorded to be -59.60 and -78.80, respectively (Table-1). Further, the values of Karl Pearson's coefficient of correlation (r) depicted in Table-1, clearly indicate that decreases in growth percent is highly dose dependent.

Observations recorded in Table-1 also show the effects of gallic acid in various combinations with NaN_3 . Antimutagenic treatments were found to be quite effective in promoting growth percentages of *R. meliloti* cultures except azide concentration at $4\times 10^{-5}\text{M}$ post treated with the two concentration levels of gallic acid. Maximum increase in the growth percent over control (-47.66) was recorded in $4\times 10^{-5}\text{M}$ NaN_3 + $1\times 10^{-4}\text{M}$ gallic acid combination and minimum value of growth percent over control (-75.11) was observed in $1\times 10^{-4}\text{M}$ NaN_3 + $1\times 10^{-4}\text{M}$ gallic acid combination.

The calculated values of 'r' at a significance level $p=0.05$ depicted in Table-1, were comparable with the tabulated 'r' values for 3 degrees of freedom, hence indicating a very strong correlation between the used concentrations of sodium azide (mutagen) and gallic acid.

Parallel to the results obtained in the present investigation, several workers have tested potentiality of various mutagens before and after antimutagenic treatments [3, 4, 5, 6] in prokaryotic systems. Thus, it could be concluded that gallic acid has the potentiality to suppress mutagenicity caused by sodium azide.

REFERENCES

- [1] M. Sumanth, and G. N. Chowdary, Antimutagenic activity of aqueous extracts of *Momordica charantia*, International Journal for Biotechnology and Molecular Biology, 1(4), 2010, 42-46.
- [2] Y. Kuroda, D. M. Shankel, and M. D. Waters, Antimutagenesis and anticarcinogenesis mechanism II, (Plenum Press, New York, London, 1990).
- [3] T. Gichner, F. Pospil, J. Veleminsky, V. Volkeova, and J. Volke, Two types of antimutagenic effects of gallic and tannic acids towards N-nitroso-compounds-induced mutagenicity in the ames *Salmonella* Assay, Folia Microbiologica, 32 (1), 1987, 55-62.



- [4] S.H. Smith, P.L Tate, G Huang, J.B. Magee, K.M. Meepagala, D.E. Wedge, and L.L. Larcom, Antimutagenic activity of berry extracts, Journal of Medicinal food, 7(4), 2004, 450-455.
- [5] S. Tangvar asittai hai, N. Sriprang, T. Harnroongroj, and S. Changbumrung, Antimutagenic activity of *Sesbania javanica* miq. Flower DMSO extract and its major flavonoid glycoside, Southeast Asian Journal of Tropical Medicine and Public Health 36(6), 2005, 1543-1551.
- [6] R. Ghazali, R. Abdullah, N. Ramli, N.F. Rajab, M.S. Ahmad-Kamal, and N.A. Yahya, Mutagenic and antimutagenic activities of *Mitragyna speciosa* Korth extract using Ames test, Journal of Medicinal Plants Research Vol. 5(8), 2011, 1345-1348.

TABLE-1: Effects of sodium azide (NaN₃; pH 6.0) alone and in combination with gallic acid on *Rhizobium meliloti* cultures.

NaN ₃ +gallic acid (M)	Optical density (OD) after 24 h or 48 h treatment Mean±SE	Growth percentage (%) x 10 ⁻⁴	Per cent decrease in growth over control
Untreated cultures	0.921±0.017	100	0
4X10 ⁻⁵ +No gallic acid	0.741±0.025	60.00	-40.00
4X10 ⁻⁵ +1X10 ⁻⁴	0.784±0.013	52.34	-47.66
4X10 ⁻⁵ +2X10 ⁻⁴	0.758±0.045	46.66	-53.11
r	-0.571	-0.805	
Untreated cultures	0.921±0.017	100	0
9X10 ⁻⁵ +No gallic acid	0.611±0.028	40.40	-59.60
9X10 ⁻⁵ +1X10 ⁻⁴	0.749±0.017	50.89	-49.11
9X10 ⁻⁵ +2X10 ⁻⁴	0.638±0.020	48.68	-51.32
r	-0.636	-0.666	
Untreated cultures	0.921±0.017	100	0
1X10 ⁻⁴ +No gallic acid	0.230±0.018	21.20	-78.80
1X10 ⁻⁴ +1X10 ⁻⁴	0.298±0.060	24.89	-75.11
1X10 ⁻⁴ +2X10 ⁻⁴	0.317±0.019	34.63	-65.37
r	-0.700	-0.671	

Tabulated r value for 3d.f. at p=0.05 is 0.878

r=Karl Pearson's coefficient of correlation.

Reported values are mean±SE of 3 replicates