



International Journal of Innovative Research in Science Engineering and Technology (IJIRSET)

(A Monthly, Peer Reviewed, Refereed, Scholarly Indexed, Open Access Journal)



Impact Factor: 8.699

Volume 14, Issue 4, April 2025

Antimutagenic effect of ascorbic acid against sodium azide induced epigenetic toxicity on seedling survival of *Trigonella foenum-graecum* L.

Dr. Manoj Kumar Rawat

Assistant Professor, Department of Botany, Samrat Prithviraj Chauhan Government College, Ajmer, Rajasthan, India

ABSTRACT: Antimutagenicity of ascorbic acid (vitamin-C) has been determined against the mutagenicity of sodium azide (NaN_3 ; pH 6.0) in *Trigonella foenum-graecum* L. using epigenetic toxicity on seedling survival percent as the parameter. Sodium azide (mutagenic agent) induces inhibitory effects on the survival percent of seedlings significantly with increasing concentrations. Sodium azide-treated series (0.0020M= 59.23%; 0.0040M= 42.00%; 0.0080M= 7.73%; 0.0100M=0%) produced a negative effect in all concentration levels when compared with the control or untreated (91%) was recorded in seeds of *T. foenum-graecum* L. Among these, the concentration level of 0.0040M was lethal dose 50 (LD 50) while the concentration level of 0.0100M was lethal because no seedling survives in this concentration level.

However, sodium azide mutagenized seeds of *T. foenum-graecum* L. were post-treated with two different aqueous concentration levels of ascorbic acid (0.0010M and 0.0100M), which showed promoting effect on seedling survival percentage compared to the control (sodium azide-treated series).

KEY WORDS: Antimutagenicity, sodium azide, ascorbic acid, *Trigonella foenum-graecum* L.

I. INTRODUCTION

Anthropogenic activities such as mining, urbanization, industrialization, and radioactive testing are at the forefront of global environmental pollution. These activities generate various substances such as E-waste, radioactive substances, a large number of physical, chemical or biological substances which include chemicals, pesticides, heavy metals, non-degradable, bio-accumulative, nutrients, biological agents and toxic substances. Most of these act as mutagens. Environmental mutagens and carcinogens can induce toxic effects in living beings. Mutagens can alter the genetic content of an organism. The effects of pollution have long term consequences. According to [1], mutations in germ line cells can be passed on to future generations, while mutations in somatic cells can lead to various pathological conditions, such as cancer. The world's greatest problem facing humanity is environmental pollution and has led to environmental causes of morbidity and mortality. The developed countries of our living planet share this burden together, however awareness and strict laws in the developed countries have contributed a great deal in protecting their environment.

It is known that the human diet contains a number of natural mutagens and carcinogens. Some of the important food mutagens identified are pyrrolizidine alkaloids, polycyclic aromatics, aflatoxins, nitrosamines, and heterocyclic amines [2, 3].

It can be reduced by antimutagens, microorganisms or plants that have biosynthetic pathways for the degradation or accumulation of environmental pollutants from soil and water. The term antimutagen was used originally to describe those agents that reduce the frequency or rate of spontaneous or induced mutations independent of the mechanisms involved [4]. Studies on antimutagenic factors (about 200 compounds) were initially carried out in the 1950s in the field of microbial genetics [5].

Ascorbic acid is a main metabolite in plants. It may act as an antioxidant and protect plants from oxidative damage resulting from photosynthesis, aerobic metabolism, and various types of environmental pollutants. The main aim of this experimental study was to raise awareness that certain substances in plants and other sources have protective effects against toxic, mutagenic and carcinogenic action of a wide range of chemical mutagens and carcinogens. For

example, Amla fruit is a rich source of antimutagens like ascorbic acid, gallic acid, and phenolic compounds and thus helps in reducing the effects of toxins entering our body and strengthening the body's immune system and digestion.

In the present study, the antimutagenicity of ascorbic acid (vitamin-C) has been determined against sodium azide (NaN_3 ; pH 6.0) induced toxicity in *T. foenum-graecum* L. with respect to seedling survival percent as the parameter.

II. LITERATURE SURVEY

The effect of acidic NaN_3 (pH 6.0) as a mutagen has been previously studied by some workers using different plant species in vivo and proven to be a potent mutagen in higher plants [6, 7, 8].

Several workers have carried out similar studies in prokaryotic system by measuring the potency of various mutagens before and after antimutagenic treatments [9, 10, 11]. Nagpal et al. [12] revealed that phenolic compounds have inhibitory effects on the toxic, mutagenic and carcinogenic activities of a wide range of chemical mutagens and carcinogens. Grover et al. [13] also pointed out that naturally occurring phenolic compounds possess a broad spectrum of biological properties and are being extensively studied for their antimutagenic/anticarcinogenic properties with the aim of ultimately using them as therapeutic/chemopreventive agents. Ames Salmonella histidine reversion assay was used by Kaur et al. [14] to evaluate the antimutagenic activity of acetonic extract of *Terminalia arjuna* on TA98 and TA100 tester strains of *Salmonella typhimurium* using direct acting mutagens (4-nitro-o-phenylenediamine, sodium azide and 2-aminofluorene). Antimutagenic or inhibitory effect of quercetin, ascorbic acid and their combination against oxidative mutagen (t-butyl hydroperoxide) was also observed by Geetha et al. [15] in Ames Salmonella assay system which also supports the present study.

III. METHODOLOGY

Dried pure line viable seeds of *T. foenum-graecum* L. were surface sterilized with 0.1% (w/v) mercuric chloride (HgCl_2) solution for 3 min. The seeds were washed thoroughly with sterilized distilled water to remove the traces of mercuric chloride and pre-soaked in distilled water for 4 h at $25 \pm 1^\circ\text{C}$.

The experiments were conducted in the following three sets-

- (1) Control or untreated-In this set, some of the pre-soaked seeds were kept in distilled water for 20 h at $25 \pm 1^\circ\text{C}$.
- (2) Treated with sodium azide (NaN_3 ; pH 6.0) alone- In this set, some of the pre-soaked seeds were treated with freshly prepared four different aqueous concentrations of sodium azide (0.0020M; 0.0040M; 0.0080M; 0.0100M) for a period of 20 h at $25 \pm 1^\circ\text{C}$.
- (3) Post-treated with ascorbic acid- In this set, some of the treated seeds (seeds treated with each concentration level of sodium azide except 0.0020M) were post treated with freshly prepared two different aqueous ascorbic acid concentration levels (0.0010M and 0.0100M) for a period of 20 h at $25 \pm 1^\circ\text{C}$.

For each set, 20 seeds were used and replicated twice. Seeds of all the experimental sets were transferred to rectangular plots in the botanical garden, Maharshi Dayanand Saraswati University, Ajmer in Rabi season. M_1 populations were regularly observed during the vegetation period. The influence of mutagen (sodium azide) alone or in combinations with an antimutagenic (ascorbic acid) was assessed in terms of seedling survival percent. The formula utilised for evaluation of data are as follows:

$$\text{Seedling survival percent (SSP)} = \frac{\text{Number of Seedlings Survived}}{\text{Total Number of Seedlings}} \times 100$$

IV. EXPERIMENTAL RESULTS

It is evident from the data depicted in Table-1 that treatment with different four concentration levels of sodium azide (0.0020M; 0.0040M; 0.0080M and 0.0100M) led to gradual reduction in the seedling survival percentage values, $59.23\% > 42.00\% > 7.73\% > 0\%$, respectively, as compared to the control (untreated) value (91%).

Maximum biological damage was occurred in 0.0100M NaN_3 treated series where no seedling survival percent was observed. Approximately 50% lethality in seedling survival percent was recorded in the 0.0040M NaN_3 treated series

where 42.00% seedling survival percent was observed. NaN_3 mutagenized seeds, post-treatment with ascorbic acid showed improved in seedling survival percent compared to the control (NaN_3 - treated series) which was in generally observed in all the treated series. Further, Karl Pearson's coefficient of correlation (r) values depicted in Table-1, clearly indicate that decrease in the seedling survival percent was highly dose dependent.

Seeds treated with only 0.0100M concentration of NaN_3 did not show any seedling survival percent but surprisingly, post-treatment with ascorbic acid, the seedling survival percent showed up to a maximum of 10%. The maximum promotion in seedling survival percent (50.66%) was observed in 0.0040M NaN_3 +0.0100M ascorbic acid treated series. However, minimum recovery in the seed germination (6.66%) was observed in 0.0100M NaN_3 +0.0100M ascorbic acid treated series.

The calculated 'r' at a significance level $p=0.05$ using parameter depicted in Table-1, with some exceptions, comparable with the tabulated 'r' values for 4 or 2 degrees of freedom ($p=0.05$), hence indicating a very strong correlation between the used concentrations of NaN_3 (mutagen) or ascorbic acid and effects on the seedling survival percent, except 0.0100M NaN_3 +0.0010M ascorbic acid and 0.0100M NaN_3 +0.0100M ascorbic acid treated series.

In the present study, after assessing the percentage average of occurrence (\bar{X}_x) values of the used parameter recorded for different treated series, it can be concluded that ascorbic acid was found to be more effective as antimutagenic (antitoxic) as per the data recorded for different combination treated series.

Table 1: The effects of sodium azide (NaN_3 ; pH 6.0) alone and in combination (post-treatment) with ascorbic acid in *Trigonella foenum-graecum* L.

NaN_3 concentrations (concentrations in molar)	Seedling survival (%)
Control	91
0.0020	59.23
0.0040	42.00
0.0080	7.73
0.0100	0
'r' Value	-0.9837
\bar{X}_x	14.51
NaN_3 (concentrations in Molar) + ascorbic acid (concentrations in molar)	Seedling survival (%)
0.0040+No ascorbic acid	42
0.0040+0.0010	46.66
0.0040+0.0100	50.66
'r' Value	0.8468
\bar{X}_x	48.28
0.0080+No ascorbic acid	7.73
0.0080+0.0010	23.33
0.0080+0.0100	29.00
'r' Value	0.7096
\bar{X}_x	22.68

0.0100+No ascorbic acid	-
0.0100+0.0010	10
0.0100+0.0100	6.66
'r' Value	0.2766*
\bar{X}_x	5.93

Tabulated 'r' for 4 d.f. at $p=0.05$ is 0.811

Tabulated 'r' for 2 d.f. at $p=0.05$ is 0.950

Reported values are mean \pm SE of 3 replicates

* Non-Significant

'r' = Karl Pearson's coefficient of correlation.

\bar{X}_x = Percentage average of occurrence

V. CONCLUSION

Conclusions can be drawn after assessing the 'r' and \bar{X}_x values for used parameter recorded for different treated series. It can be concluded that sodium azide was observed to be more toxic.

Similarly, based on the statistical analysis (Karl Pearson's coefficient of correlation (r) and \bar{X}_x values), it can be concluded that ascorbic acid has the potential to recover biologically damaged seedling survival in azide mutagenized seeds of *T. foenum-graecum* L.

REFERENCES

- [1] Sloczynska, K., Powroznik, B., Pekala, E. and Waszkielewicz, A.M. (2014), "Antimutagenic Compounds and their possible Mechanisms of Action", J. Appl. Genet., 55: 273–285.
- [2] Matthew, D. W. and Fradet-Turcotte, A. (2018), "Virus DNA Replication and the Host DNA Damage Response", Annu. Rev. Virology, 5: 141–164.
- [3] Cellai, F., Capacci, F., Sgarrella, C., Poli, C., Arena, L., Tofani, L., Giese, R.W. and Peluso, M. (2019), "A Cross-Sectional Study on 3-(2-Deoxy-d-Erythro-Pentafuranosyl) Pyrimido[1,2] Purin-10(3H)-One Deoxyguanosine Adducts among Woodworkers in Tuscany, Italy", Int. J. Mol. Sci., 20: 2763.
- [4] Novick, A. and Szilard, L. (1952), "Antimutagens", Nature, 170: 926-927.
- [5] Clarke, C. H. and Shankel, D. M. (1975), "Antimutagenesis in Microbial Systems", Bacteriology Review, 39 (1), 33-53.
- [6] Al-Qurainy, F. and Khan, S. (2009), "Mutagenic Effects of Sodium Azide and its Application in Crop Improvement", World Applied Sciences Journal, 6 (12): 1589-1601.
- [7] Rawat, M.K. and Meena, R.P. (2013), "Evaluating Antimutagenic Effects of Vitamin-C and L-Cysteine alone and in combination towards Sodium azide-Induced Toxicity in *Trigonella foenum-graecum* L.", South Asian Journal of Experimental Biology, 3(3):127-130.
- [8] Alhadi, R. A. and Aabou-Isba, S. M. (2022), "Effect of Different Concentrations of Sodium Azide on Some traits of Barley (*Hordeum vulgare* L.)", Acta Scientific Agriculture, (6)-8.
- [9] Ghazali, A. R., Abdullah, R., Ramli, N., Rajab, N. F., Ahmad-Kamal, M. S. and Yahya, N. A. (2011), "Mutagenic and Antimutagenic Activities of *Mitragyna speciosa* Korth Extract Using Ames test", Journal of Medicinal Plants Research, 5(8): 1345-1348.
- [10] Makhafola, T. J., Elgorashi, E. E., McGaw, L. J., Verschaeve, L. and Eloff, J. N. (2016), "The Correlation Between Antimutagenic Activity and Total Phenolic Content of Extracts of 31 Plant Species with High Antioxidant Activity", BMC Complementary and Alternative Medicine, 16:490.
- [11] Rehman, S. (2021), "Antimutagenic Effect of Medicinal Plants from Alternative Medicine", International Journal of Pharmacognosy & Chinese Medicine, 5 (1).

- [12] Nagpal, A., Grover, I. S., Arora, S. and Kaur, H. (2000), “Anticlastogenic Effects of Acid Methanol Fraction of Terminalia arjuna Roxb. in Root Tip Cells of Allium cepa L.”, Environmental Protection, Thukral, A. K. and Virk, G. S. eds Scientific Publishers, Jodhpur, India, 275-282.
- [13] Grover, I. S. Nagpal, Arora, A., Kaur, S. and Sharma, A. (2000), “Antimutagenic Effect of Ether Extract of Terminalia arjuna in Ames Assay”, Environmental Protection, Thukral, A. K. and Virk, G. S. eds. Scientific publishers, Jodhpur, India, 263-267.
- [14] Kaur, S., Kumar, S., Kaur, P. and Chandel, M. (2010), “Study of Antimutagenic Potential of Phytoconstituents Isolated from Terminalia arjuna in the Salmonella/Microsome Assay”, American journal of biomedical sciences, 2(2):164-177.
- [15] Geetha, T., Malhotra, V., Chopra, K. and Kaur, I. P. (2005), “Antimutagenic and Antioxidant / Prooxidant Activity of Quercetin”, Indian Journal of Experimental Biology, 43: 61-67.



INTERNATIONAL
STANDARD
SERIAL
NUMBER
INDIA



INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH

IN SCIENCE | ENGINEERING | TECHNOLOGY

 9940 572 462  6381 907 438  ijirset@gmail.com



www.ijirset.com

Scan to save the contact details