JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Effect on Allium cepa and Allium sativum by organic substance of 1,2,4,5-tetrazin

Smita S. Kharkale-BhuyarAssistant
Professor
Shri Lemdeo Patil Mahavidyalaya, Mandhal, Kuhi, Nagpur, Maharashtra, India

Abstract: Study investigated effect on Allium sativum L. (garlic) and Allium cepa L. (onion) by organic substance of 1,2,4,5-tetrazin were analyzing root chromosomal abnormalities. The root tips of the two plant species were treated with different concentrations (0.001M, 0.002M, 0.003M) of 1,2,4,5-tetrazin mixed with 10% Dimethyl Sulfoxide-Water. After treatment, various types of physiological hydrolyze, squashes, mitotic index (MI), cytological abnormalities, and chromosomal aberrations were scored. The results indicated a genotoxic effect, with a significant reduction in the mitotic index found to be dose-dependent. The findings suggest that 1,2,4,5-tetrazin has a toxic effect on the root cells of garlic and onion, and that the magnitude of the effect increases with increasing concentration of the chemical.

Keywords: Mitotic index, genetoxicity, root-shoot ratio, Chromosomal aberrations.

Introduction

Among all crops, onion and garlic are two important crops worldwide with a production of 26.94 million metric tons of garlic and 4.46 million metric tons of onion. And second ranks in India region 13.20 lakhha and manufacture 209.31 lakh tones of onion and in garlic, too second in zone 2.81 lakh ha and manufacture 6.17 lakh tonnes, next to China. In India 30 to 36species have been reported[1-3]. This depicts an increased consumption in the recent years due to the expansion of the Mediterranean and Asian cuisine. While, presence confidential as vegetables, due to their strange taste and smell, they are majorly existence used in cooking presentations. Together onion and garlic have great demand assigning to their remedial and beneficial ideals in both customary as well as current drug.

Considering the contemporary existence and position of these corm harvests in everyday lifecycle, onion and garlic are being treated into dissimilar lubricants, pastes, powders, pickles, and extracts. All these processed forms of onion and garlic pronounce different properties as well as a significant difference in the properties and quantities of their bioactive compounds. In 1,2,4,5-tetrazines have involved abundant consideration due to

their diverse medicinal,

biology, industrial and agricultural importance[4-5].

These compounds act as fungicide, insecticide, and anticonvulsant. It is used in antibiotic, bactiostatic, sedative and non-nutritive sweetener. They have been used as antiviral, antituberculosis and antisporiatic agents [6]. They are also used in the treatment of malaria. These are also useful in fighting fungiform and microbial contagion on plants. 1,2,4,5-tetrazines are show anticonvulsant, antiinflammatory, insecticidal, fungal, analgesic antitumor properties, herbicidal activities [7-10] and antimalarial activities. Therefore, an attempt is made to investigate in some detail the structure ramifications of this apparently unique system. The mutagenicity and genotoxicity evaluation, the ames test is the most commonly usedgenotoxicity assay in regulatory toxicology. The present challenge is to evaluate the genotoxic effects of 1,2,4,5 - tetrazine. In overall, chromosome mutilation is deliberated as a quantity of hereditary hazards, which has been observed to be reliable index. The conditions for determinate of genotoxicity were obvious by measurable rapport among biological experience and mitotic conflicts. The hereditary changes recorded in present-day study were comparative abnormality rate, chromosome fragment, laggared formation, Chromosome Bridge, sticky metaphase and anaphase [11-13]. Among various plant bio-assays, chromosomal aberration assay in Allium sativum root is one of the most It has been known that 1,2,4,5-tetrazin causes chromosomal damage in root cells of plants.

Accordingly, recently certain researches have been suggested that the chemicals that are commonly added to pesticides may change the activity of pesticides (Holland et al. 2002). The Allium test is a very good plant bioassay for chromosome damage both in mitosis and meiosis and for somatic mutations induced by chemicals and radiations. The present study was aimed to determine the effect of 1,2,4,5-tetrazin on the process of mitosis on Allium cepa L. and Allium sativum L. by using Allium test.

2. Materials and Methods

For the genotoxicity, plant materials used for test were Allium cepa L. (2n=16) and Allium sativum L. (2n=16). Clean, healthy bulbs and equal sized of Allium cepa L. (onion bulbs) and Allium sativum L. (garlic bulbs) were chosen and allowed to germinate in glass container containing distilled water for 24 hours at 32 °C until the root-length reached about (0.3-0.4 cm).. Before starting to the experiments, dry scales of bulbs were removed and 0.001M, 0.002M, 0.003M, concentrations of 1,2,4,5-tetrazin with 10% Dimethyl Sulfoxide-Water were used. The solutions were prepared in distilled water. Allium cepa L. and Allium sativum L. roots were treated with different concentrations of 1,2,4,5- tetrazin. Controls were treated with distilled water for the same time periods. The biological material was fixed with a mixture of absolute ethyl alcohol and glacial acetic acid in a volume ratio of 3:1 for 24 h. The roots were hydrolyzed in 1N HCl at 60°C for 5 min, and then stained by acetocarmine stain.

About 2 to 3 mm fatal root tips were cut off using a sharp blade, and then placed on a spotless slide with acetocarmine drop. The slide was covered with coverslip on the root tip and was squashed by applying uniform pressure. The slides were examined under the light microscope. Photomicrographs were taken with Sony digital camera, directly from eyepiece of microscope[14-16].

3. Statistical Analyses

The mitotic index was calculated using the following formula:

$$MI\ (\%) = \frac{\text{Total number of cells in division}}{\text{Total number of analysed cells}} \times 100$$

The index of the total abnormalities (TAs) was also calculated:

$$TA\ (\%) = \frac{\text{Total number of aberrant cells}}{\text{Total number of cells in division}} \times 100$$

4. Results

All of the concentrations of tetrazin with 10% Dimethyl Sulfoxide-Water used in the present study induced important abnormalities during mitotic division when compared to control in both Allium cepa L. and Allium sativum L. These abnormalities were: c-Mitosis, chromosome stickiness, anaphase and telophase bridges, laggard chromosomes, multipolar anaphase, micronuclei and fragments. The highest abnormality number was observed in root tips of Allium

ativum L. in 0.003M concentration while the highest abnormality number was seen in root tips of Allium cepa L. in 0.002 concentration. The results obtained from control series and processed plants are shown in Table. Distributions of abnormalities according to stages were different. The most frequent abnormalities were seen at metaphase and at this stage the predominant type of abnormality was c-Mitosis. The examples of chromosomal abnormalities induced by different concentrations and treatment periods of tetrazin with10% Dimethyl Sulfoxide-Water. The mitotic index reflects the frequency of cell division. In the present study, the mitotic index was seen to decrease with increasing concentration of tetrazin with 10% Dimethyl Sulfoxide-Water concentrations in both plants. The complete inhibiton was seen at 24 hrs treatment period.

Treatment of allium sativum roots with different concentration of 0.001M, 0.002M, 0.003M of 1,2,4,5-tetrazin with10% Dimethyl Sulfoxide-Water revealed different types of chromosomal aberrations and cytological abnormalities such as mitotic index anaphase, metaphases, bridges, laggards, micronuclei and stickiness. In dissimilar phases of mitosis, the crush provisions of root tip cells of negative control sections exposed a great number of usual dividing cells. Though, later root tips treated with unlike attentions of 1,2,4,5-tetrazin with10% Dimethyl Sulfoxide-Water were dense, a number of separating cells with diverse kinds of deviations such as c- mitosis, disturbed anaphases, stickiness, laggards, disturbed anaphases, abnormal metaphases, bridges and chromosomal breaks were observed.

carbofuran, allium sativum presentation to carbofuran for 24 hrs of destructiveness was unveiled that chromosomal and mitotic deviations in the root meristem cells.[19]. chromosomal trouble were resolute such as micronucleus, Hyperchromasia, later segregation, c-mitosis, chromosome loss, pulverised nucleus, chromosomal adherence, chromatin globules. According to consequences, the total chromosomal altering enhanced through budding quantities of 1,2,4,5-tetrazin with 10% Dimethyl Sulfoxide-Water and the switch as associated to exposure time[17-22].

In current reading, genotoxic altering was determined intracellular levels. At this time Outcomes attainable approve the largest sensitivity of root with considerable upsurges with cell disfigurement attraction of cell through doses of 24-72 hrs [23] of increasing doses of 1,2,4,5-tetrazin with 10% Dimethyl Sulfoxide-Water.

Main cellular variable was establish through mode of dissimilar types of deviations such as c-mitosis, disturbed anaphases, stickiness, laggards, disturbed anaphases, abnormal metaphases, bridges and

chromosomal interruptions were detected. Sub-chromatid bridges mean impolite folding of the chromosomal gossamers which have caused in combination of threads and genes become attach to each other [24-25]. In the current effort, in whole absorptions of 1,2,4,5 - tetrazine, has been originate stickiness. The maximum concentration of stickiness was detected at the maximum concentration of 1,2,4,5 - tetrazine.

5. Conclusion

The genotoxic effect of 1,2,4,5-tetrazin using Allium sativum L. [Garlic] and Allium cepa L. [Onion] root chromosomal abnormality analyzed. These results show that the effect of 1,2,4,5-tetrazin on roots cell be contingent on the concentrations and times. Consequently, it would be important to more contaminated outcome and cytological indications at molecular level to define the reliable unclean effect of 1,2,4,5-tetrazin.

Table 1: Mitotic index and the percentage of mitosis in the root tip cell of Allium sativum (garlic)] treated with different concentration 0.001M, 0.002M and 0.003M of 1,2,4,5-tetrazin with 10% Dimethyl Sulfoxide-Water (DMSO)

Sr. No.	Treatment	Mitotic Index	% Prophase	% Metaphase	% Anaphase	% Telophase
1	Negative control	9.3580	51	33	12	10
2	Positive control	6.1251	0	0	0	0
3	0.001M	1.0273	21	20	9	5
4	0.002M	1.133	23	23	11	7
5	0.003M	1.162	27	28	14	10

Table 2: Mitotic aberration in root tip cells of Allium sativum (garlic) treated with 0.001M,0.002M, 0.003M of1,2,4,5-tetrazin with 10% Dimethyl Sulfoxide-Water (DMSO)

Sr. No.	Treatment	% total Aberrant Cells	% Prophase	% Metaphase	% Anaphase	% Telophase
1	Negative control	0	---	--	--	--
2	Positive control	0	---	--	--	--
3	0.001M	3.232	4.2	25.45	--	--
4	0.002M	3.323	6.2	28.20	0.5	0.1
5	0.003M	3.333	7.8	33.33	0.7	0.4

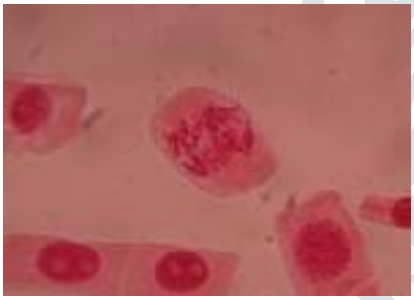
Table 3: Mitotic index and the percentage of mitosis in the root tip cell of Allium cepa L. [Onion] treated withdifferent concentration 0.001M, 0.002M and 0.003M of 1,2,4,5-tetrazin with 10% Dimethyl Sulfoxide-Water (DMSO)

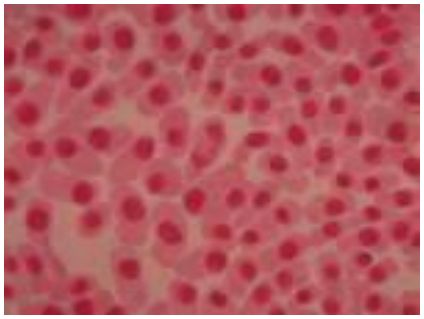
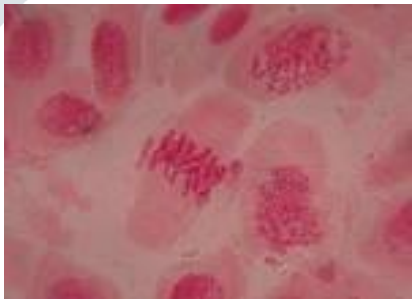
Sr. No.	Treatment	Mitotic Index	% Prophase	% Metaphase	% Anaphase	% Telophase
1	Negative control	6..9953	47	33	12	10
2	Positive control	6.5870	0	0	0	0
3	0.001M	1.325	15	16	5	2
4	0.002M	1.584	21	22	8	4
5	0.003M	1.989	27	25	11	7

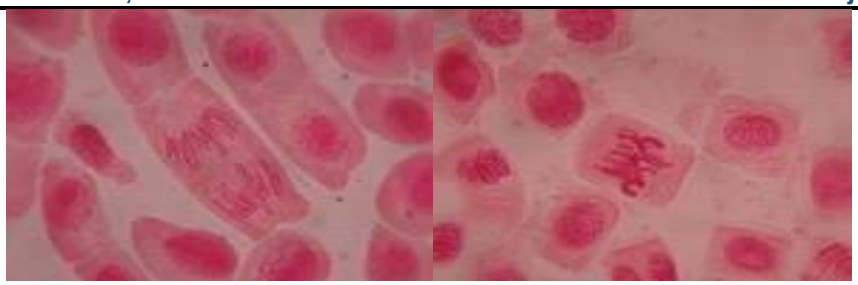
Table 4: Mitotic aberration in root tip cells of Allium cepa L. [Onion] treated with 0.001M,0.002M, 0.003M of1,2,4,5-tetrazin with 10% Dimethyl Sulfoxide-Water (DMSO)

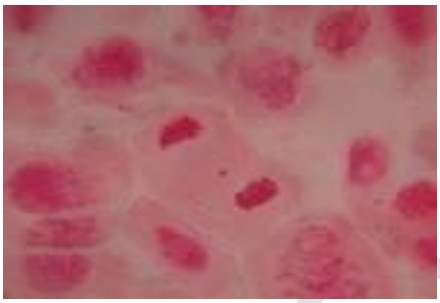
Sr. No.	Treatment	% total Aberrant Cells	% Prophase	% Metaphase	% Anaphase	% Telophase
1	Negative control	0	---	--	--	--
2	Positive control	0	---	--	--	--
3	0.001M	3.02	3.9	22.45	--	--
4	0.002M	3.21	7.8	29.40	0.1	0.2
5	0.003M	3.53	9.2	34.33	0.3	0.5

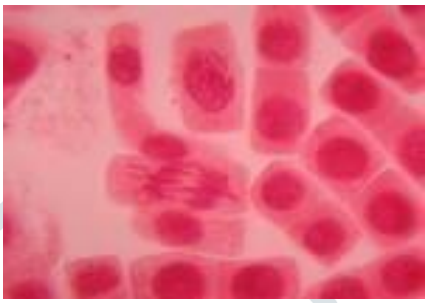
The genotoxic effect of 1,2,4,5-tetrazin with10% Dimethyl Sulfoxide-Water using Allium sativum L. [Garlic] andAllium cepa L. [Onion] Plate I- Anaphase, Plate II-Bridge, Plate III-Disturded Metaphase, Plate IV-laggard, Plate V-Metaphase, Plate VI-Precocious, DP: Disturbed prophase. DM: Disturbed metaphase. DA : Disturbed anaphase, B: Bridge

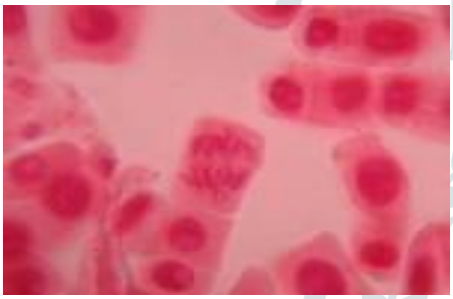


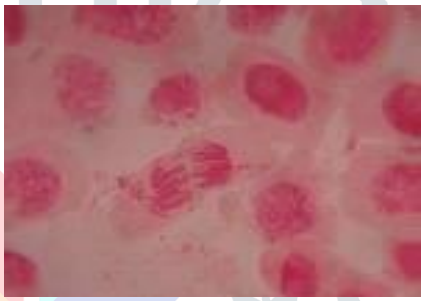













6. Referances

1. Alvarez M.C., L.A. Santerre, G.G. Zuniga, B.O. Torres, C.E. Padilla and V.A. Feria, 2001- Evaluation of genotoxic activity of Maleic hydrazide ethly methane sulfonate and N-Nitroso diethylamine in Tradescantia using the commey assay. Salud Publica de Mexico, 43: 6-12.

2. Gomurgen A.N., 2000 — Cytological effect of the herbicide 2,4-D isooctylester 48% on root mitosis of Allium cepa. Cytologia, 65: 383-388.

3. Hidalgo A., R.J.A. Gonzales, P. Navas and H.G. Garcia, 1989-Abnormal mitosis and growth inhibition in Allium cepa roots induced by propham and chlorpropham. Cytobios, 57: 7-14.

4. Holland N.T., P. Duramad, N. Rothman, L.W. Figgs, A. Blair, A. Hubbard and M.T. Smith, 2002- Micronucleus frequency and proliferation in human lymphocytes after exposure to

herbicide 2,4- Dichlorophenoxy acetic acid in vitro and in vivo. Mutation Research, 521: 165-178.

5. Kharkale S.S., 2021- Valuation of Genotoxic Effect of Aqueous Organic Compound withAllium Sativum [Garlic], IJARSCT, 12: 47- 52.

6. Saxena P.N., Gupta S.K., Murthy R.C. 2010-Carbofuraninducedcy to genetic effects in root meristem cells of Allium cepa and Allium sativum: A spectroscopic approach for chromosome damage. Pesticide Biochemistry and Physiology. 96:93–100.

7. Kim J.Y., Kwon O. 2009- Garlic intake and cancer risk: An analysis using the Food and Drug Administration 'sevidence-based review system for the scientific evaluation of health claims. AmJ Clin Nutr, 9:257-64.

8. Agarwal S.S., Singh V.K. 1999-Immunomodulators: A review of studies on Indian medicinal plants and synthetic peptides part I; medicinal plants. Proceedings of the Indian National science academy, 65:179-204.

9. Kovalchuk I.; Kovalchuk O. 2008-Transgenic Plants as Sensors of Environmental Pollution Genotoxicity. Sensors (Basel), 8, 1539–1558.

10. Bakadir, K., Kassale, A., Barouni, K., Lakhmiri, R., Albourine, A.J. 2016- Retention of a compound of herbicides, 2,4-dichlorophenoxy acetic acid, to a soil in the absence and in the presence of Cu(II) and Zn(II) cations. J. Mater. Environ. Sci. 7, 1056–1063.

11. Nefic, H., Musanovic, J., Metovic, A., Kurteshi, K. 2013-Chromosomal and nuclear alterations in root tip cells of Allium Cepa L. Induced by Alprazolam. Med. Arch. 67, 388–392.

12. Silveira, G.L., Franco Lima, M.G., Barreto dos Reis, G., Palmieri, M.J., Fonseca Andrade- Vieria, L. 2017- Toxic effects of environmental pollutants: Comparative investigation using Allium cepa L. and Lactuca sativa L.Chemosphere, 178, 359–367.

13. Bonciu E., Firbas P., Fontanetti C.S. Wusheng, J., Karaismailo˘ glu, M.C., Liu, D.; Menicucci, F., Pesnya, D.S., Popescu, A., Romanovsky A.V. - 2018 evaluation for the standardization of the Allium cepa test as cytotoxicity and genotoxicity assay, Caryologia, 71, 191–209.

14. Mustafaaand, Y. and Arikan, E.S. (2008) Genotoxicity Testing of Quizalofop-P-Ethyl Herbicide Using the Allium cepa Anaphase-Telophase Chromosome Aberration Assay. Caryologia, 61, 45-52.

15. Sinkkonen, A., Myyrä, M., Penttinen, O.-P. and Rantalainen, A.-L. (2010) Selective Toxicityat Low Doses: Experiments with Three Plant Species and Toxicants. Dose-Response, 9, 130-143.

16. Akyil, D., Oktay, S., Liman, R., Eren, Y. and Konuk, M. (2012) Genotoxic and Mutagenic Effects of Aqueous Extract from Aerial Parts of Achillea teretifolia. Turkish Journal of Biology, 36, 1112-1125.

17. Iwalokun, B.A., Oyenuga, A.O., Saibu, G.M. and Ayorinde, J. (2011) Analyses of Cytotoxic and Genotoxic Potentials of Loranthus micranthus Using the Allium cepa Test Current Research.Journal of Biological Sciences, 3, 459-467.

18. Rathnasamy, S., Mohamed, K.B., Sulaiman, S.F. and Akinboro, A. (2013) Evaluation of Cytotoxic, Mutagenic and Antimutagenic Potential of Leaf Extracts of Three Medicinal Plants Using Allium cepa Chromosome Assay. International Current Pharmaceutical Journal, 2, 131- 140.

19. Marcano L., I. Carruyo, A. Del Campo and X. Montiel, 2004 — Cytotoxicity and made of action of Maleic hydrazide in root tips of Allium cepa L.. Environmental Research, 94: 221-226.

20. Patil B.C. and B.I. Bhat, 1992 — A comparative study of MH and EMS in the induction of chromosomal aberrations on lateral root meristem in Clitoria ternetea L.. Cytologia, 57: 259-264.

21. Rank J., L. Lopez, H. Mette and J. Moretton, 2002 — Genotoxicity of maleic hydrazide, acridine and DEHP in allium root cells performed by two different laboratories. Hereditas, 136: 13-18.

22. Reddi T.V.V.S. and V.R. Reddi, 1985 — Cytological effects of chemical mutagens in rice. Cytologia, 50: 499-405.

23. Marcano L., I. Carruyo, A. Del Campo and X. Montiel, 2004 — Cytotoxicity and made of action of Maleic hydrazide in root tips of Allium cepa L.Environmental Research, 94: 221-226.

24. Patil B.C. and B.I. Bhat, 1992 — A comparative study of MH and EMS in the induction of chromosomal aberrations on lateral root meristem in Clitoria ternetea L.. Cytologia, 57: 259-264.

25. Gomurgen A.N., 2000 — Cytological effect of the herbicide 2,4-D isooctylester 48% on root mitosis of Allium cepa. Cytologia, 65: 383-388.