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THE EVALUATION OF VARIOUS ORGANIC SOLVENT USING *ALLIUM CEPA* TEST

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ABSTRACT
In the present work the simplified allium cepa L. root assay was utilized to evaluate the possible cyto and genotoxic effect of dihydroformazan (S1) and 1,2,4,5-tetrazin (S9). Allium cepa L. Was treated with 0.001M dihydroformazan (S1) and 1,2,4,5-tetrazin (S9) in 10% DMF-Water were used in the present investigation. After treatment, the root were hydrolyzed, squashed, mitotic index (MI), cytological abnormalities and Chromosomal aberrations rate were scored. Subsequently the results exposed a genetoxicity effect as well as significance reduction in the Mitotic index. Chromosome stickiness suggest a toxic effect S1 and S9. In conclusion, our study indicates that the effect on root tips of allium cepa L. Was more effective for contravention DNA linkage in dihydroformazan (S1) than 1,2,4,5-tetrazin (S9) which may be due more basic free –NH2 end.

KEYWORD: Allium cepa L., Chromosomal aberrations, Mitotic index, 1,2,4,5-tetrazin.

INTRODUCTION
Studies on the mechanism of the anticancer introduction development have given away that specific chemical compounds are able to restore to health diseases by themselves. The probable environmental and human health impact connected with the heavy use of organic compounds, the dihydroformazan (S1) and 1,2,4,5 – tetrazins (S9) were assessed for reserve of cell division (cytoxicity) and genotoxicity using the *Allium cepa* chromosome abnormality attempt.[1-3]

Substituted dihydroformazan (S1) and 1,2,4,5 – tetrazin (S9) constitute potentially useful class of organic compound for pharmaceutical applications, as it possesses a diverse range of physiological activities.[4-8] Various substituted S1 and S9 were shown to exhibit plant growth promoting activity.[9] Their use as anticancer, antimicrobial, antimalarial was also studied.[10-11] Several workers[12] reported the synthesis of dihydroformazan (S1) and 1,2,4,5 - tetrazin are herbicide and it's used as pesticides have also been reported.[13] Such a widespread use of S1 and S9 in the biological purposes obligation to concentrate on its activity on plant growth, ultimate on cell division.

The root tips several of palnt diversity have been used for the study of induced chromosomal aberrations (CAs) and presence of micronuclei (MNi). Dissimilar allium species root tips are used the most abundant as experimental material. Alliums species acquire some advantages over other organism in convinced situation. Plant systems had a most important part in near the beginning investigations of the genetic changes caused by mutagenic chemical and radiation. The first modification of the allium cepa test for environmental monitoring was introduced by Fiskesjo.[14-18]

The species of allium cepa L. Present advantages, including low raise cost, easy handling and suitable chromosomal features. This plant bears large and few chromosomes (2n=16), which facilitates the evaluation of chromosome damages and disturbances in cell division cycle.[19] Root tips of Allium cepa have been suggested as a standard for cytogenetic evaluate in environmental monitoring due to the rectification of these plant life with mammalian and non-mammalian test system.

The mutagenicity and genotoxicity evaluation, using more than one test system, is often pointed out. The present attempt is to assess the genotoxic effects of dihydroformazan (S1) and 1,2,4,5 – tetrazin (S9). In general, chromosome damage is considered as a measure of genetic hazards, which has been observed to be reliable index. The criteria for determinate of genotoxicity were decided by quantitative relationship between chemical exposure and mitotic disturbances. The genetic alterations scored in present investigation were relative abnormality rate, chromosome fragment, laggared formation, Chromosome Bridge, sticky metaphase and anaphase.

Structures of Dihydroformazan (S1) and 1,2,4,5 – tetrazin (S9) In dihydroformazan containing free –NH2

group which makes it more basic while in 1,2,4,5 – tetrazin (S9) all the four – N are present in a six membered ring and also – CH3 is present as one of the substituent of aromatic ring where as no substitution present in dihydroformazan (S1).

MATERIALS AND METHODS

Chemicals

In this study, it was used 0.001M dihydroformazan (S1) and 1,2,4,5-terazin prepaed in 10% DMF- Water were used in present investigation.

Root Tips of Allium cepa L.

The plant system was Allium cepa L.,2n = 16 commonly known as onion were obtained commercially and were placed in small jars with the basal ends dipping in distilled water. The new emerged roots (1-2 cm in length) were treated with a 0.001M dihydroformazan (S1) and 1,2,4,5-terazin prepaed in 10% DMF- Water. Treatments took place in the dark. A negative control has been treated with distilled water. After 24h, the roots of each bulb were extracted and fixed in freshly prepaed and cool mixture (4-10 oC) containg three parts of methanol and one part of glacial acetic acid (3:3, v/v) for 24h. Roots can be stored in fixative for several days or weeks.

For preparation root tip chromosome slides acetorcein squash technique has been used to analyse mitotic index, cytotoxic effects and CAs. The root tips of bulbs hydrolysed in 1N hydrochloric acid (HCl) at 600C for 4-5 min. The cell wall has to dissolve by hydrolysis with acid. From HCl the root are transferred to distilled water and left for few minutes. The roots then were transferred on clean slide. Root tips were used for each slide. On the slide, tips were crushed in drop of 2% acetorcein[20] with the flat end of metal rod (taper) and squashed under a cover slip. The pressure was applied under several thickness of bloting filter paper during sideways movements of cover slip must be avoided. The two slides prepared for each of bulbs in each experimental group. Fifteen micro preparations of each sample and controls. For cytogenetic examination of mitotic cells was used a microscope at 1000*magnification. The mitotic index was calculated for each treatment as a number of dividing cells/100 cells. The cytological abnormalities such as anaphase briges, laggards, micronuclei and stickiness were scored in the mitotic cells.

RESULTS AND DISCUSSION

The mitotic index and consequently, the frequency of mitotic phase are given in table 1 for cells of Allium cepa treatments with concentration 0.001M of dihydroformazan (S1) and 1,2,4,5-tetrazin (S9) with 10% DMF-Water.

The aberration frequency with S1 and S9 was found to be 2.45 and 2.01, respectively. However, the effect produced by these compounds in dividing cells varied significantly. It seems that both of these compounds promoted the premature shortening of kinetochore microtubules. This is evident from very high frequency (more than 50%) of precocious movement of chromosomes during mitosis. In case of S9 next most common aberration was found to be disturbed prophase with frequency of 27.3%, which results due to insufficient condensation of chromosomes. Laggards were found with the frequency of 4.6% in case of S9, which is suggestive of either dissolution of spindle fibers or their failure to attach themselves to centromeres. On the other hand S1 was found to have more diverse effect than S9. After precocious movement, chromosome bridges were most common (with frequency of 22.2%) in case of S1. Bridges are formed in case of dicentric chromosomes, which are result of either chromosome splitting or fusion of two centric fragments of the chromosome. In addition to this, laggards, disturbed prophase, disturbed metaphase and disturbed anaphase were also observed in case of S1 with frequency of > 10.0 (Table 1 and Table 2).

The chromosomal aberrations seen in the present study are the result of, either singly or in combination, spindle dissolution, changes in the constitution of chromosomes like depurination and hydrolysis of phosphate bonds of the double helix, depolymerization of nucleic acids, changes in the viscosity of cytoplasm and failure of repair mechanism.[21-24] This indicates the effect of S1 and S9 on DNA and/or protein structure and function. It is well known that chemical induced chromosome breaks are temporally and spatially localized and moreover, they have regional specificity. It should be noted that in S1, presence of free – NH2 group makes it more basic as compared to S9 where all the four –N are present in a six membered ring. Hence, S1 is somewhat more reactive than S9, which is evident from slightly high frequency of chromosomal aberrations in case of S1. Moreover, S9 has an electron releasing –CH3 group as one of the substituent of aromatic ring where as no substitution is present in S1. This presence of –CH3 group further decreases the possibility of nucleophilic substitution reaction, thereby making it less reactive.

Bridges, which are the result of formation of dicentric chromosomes due to chromosomal breaks or splitting[25-26], were seen in the samples treated with S1. The chromosome break occurs due to hydrolysis of phosphate backbone of DNA.[27] In S1 free –NH2 group is in conjugation with ring, which gives the possibility of donating lone pair of electrons of –NH2 group to the phosphate group thus breaking the phosphate backbone of DNA. In addition to this S1 is aliphatic tertiary amine and hence there are more sites available for donating lone pair of electrons to P in phosphate ester.

The other types of aberrations like, precocious movement, laggards etc. seen in the samples are due to interference with protein structures where peptide bonds are predominantly present in addition to hydrogen and SH bonds. Both the compounds i.e. S1 and S9 have

groups which can react with the amino acids and the bonds joining them. Thus, the treatment of S1 and S9 might have interfered in the structure and function of microtubules and other proteins including the enzymes involved in DNA repair; by reacting with them.

Thus, from the present study we conclude that S1 and S9 can have a profound effect on the biota of ecosystem due to their genotoxic properties. These compounds affect the cell division, which is an important aspect of growth and development of any living entity. Thus, a detailed investigation is needed to assess the biosafety of these compounds. In addition to this, safe disposal practices are needed to reduce the environmental hazards posed by these chemicals. Moreover, we also recommend a detailed investigation of dihydroformazan and 1,2,4,5-tetrazine and their derivates before being used as herbicide.

Table1: Mitotic index and the percentage of mitosis in the root tip cell of Allium cepa treated with 0.001M of dihydroformazan (S1) and 1,2,4,5-tetrazin (S9) with 10% DMF-Water.

Sr. No.	Treatment	Mitotic Index	% Prophase	% Metaphase	% Anaphase	% Telophase
1	Negative control	8.880.21	45	25	12	09
2	Positive control	7.74.55	0	0	0	0
3	S1	1.120.99	15	20	12	23
4	S9	1.56	21	25	8	7

Negative control: distilled water. Positive control: 10% DMF-Water solution.

Table. 2: Mitotic aberration in root tip cells of Allium cepa treated with 0.001M of dihydroformazan (S1) and 1,2,4,5-tetrazin (S9) with 10% DMF-Water.

Sr. No.	Treatment	% total Aberrant Cells	% of Abberations					
			PM`	L	DP	DM	DA	B
1	Negative control	0	-	-	-	-	-	-
2	Positive control	0	-	-	-	-	-	-
3	S1	2.45	55.6	3.7	7.4	7.4	3.7	22.2
4	S9	2.01	4.6	27.3		0	0	0

Negative control: distilled water. Positive control: 10% DMF-Water solution. PM: Precocious movement, L: Laggards, DP: Disturbed prophase. DM: Disturbed metaphase. DA : Disturbed anaphase, B: Bridge

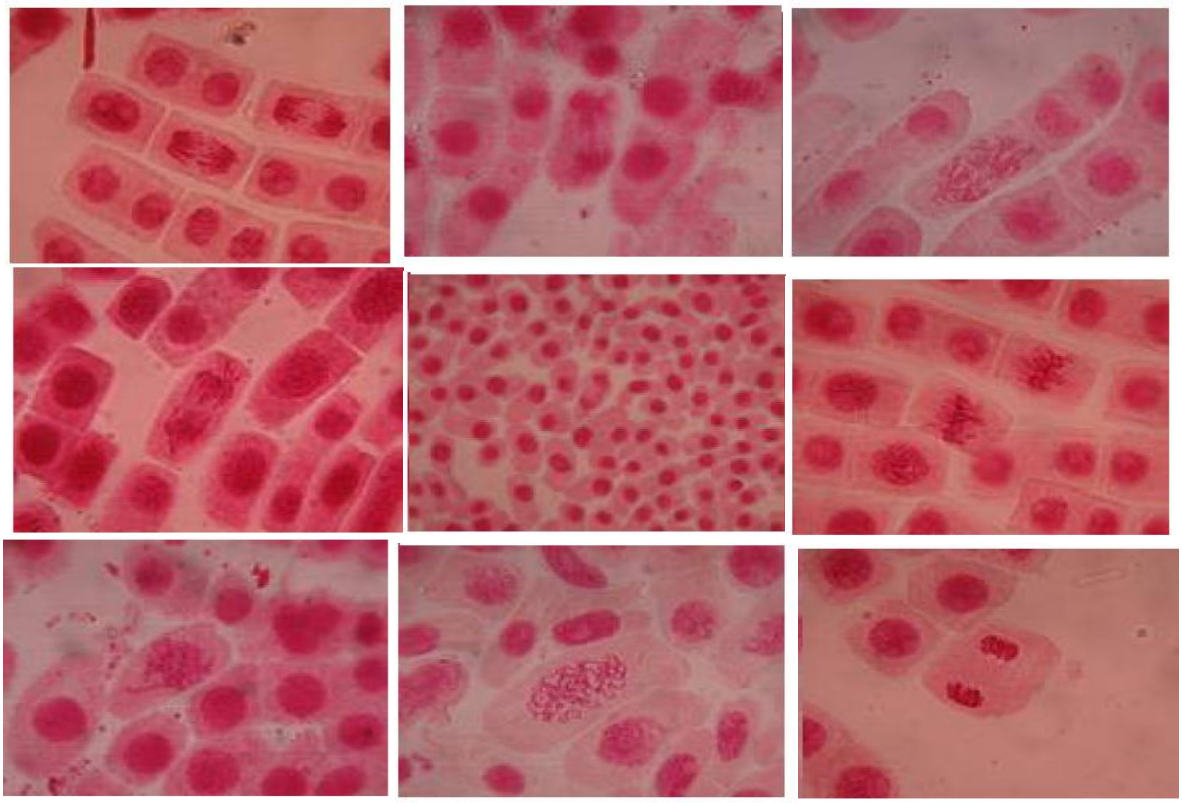

Plate I- Anaphase, Plate II-Bridge, Plate III-Disturded Metaphase, Plate IV-laggard, Plate V-Metaphase, Plate VI-Precocious, Plate VII- Precocious and Bridg, Plate VIII- Prophase, Plate Ixtelophase

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REFERENSES

1. Wiley PF, The Chemistry of Heterocyclic Compounds, The 1,2,4-Triazines, 1,2,4,5 – Tetrazines and Pentazines. A.Weissberger, Ed., Interscience, New York, 1956; 16-881.
2. Thompson PE, Werbel LM, Antimalaria Agents, Chemistry and Pharrmacology, Academic Press, New York, N.T. 1972; (16): 885.
3. Ziman SD, Heterocycles from Substitued Semicarbazides and Thionyl Chloride, J. Hetrerocyclic Chem, 1979; (16): 895.
4. Werbel, LM, McNamara DJ, Colbry NL, Synthesis and Antimalarial Effects of N,N-Dialky – 6 – (sub.phenyl) – 1,24,5- tetrazin - 3 amines(1,2), J. Hetrerocyclic Chem, 1979; (16): 881.
5. Rothgery EF, Scott RN, Use of Selected 1,2 – Dihydro – 1,2,4,5 – tetrazines as Oxygen – Scavenging Agents.United State Patent, 1983; (21): 1-7.
6. International Agency for Research on Cancer (IARC), Monographs on the Evalution of Carcinogenic Risk to Humans, Occupational Exposures in Insecticide Application and Some Pesticides. IARC, Lyon, 1991.
7. Thais C, Dania EC, Maria A, Pesticide Biochemistry and Physiology. Mechanism of micronuclei formation in Polyploidizated cells of Allium cepa exposed to trifluralin herbicides, 2007; 3(88): 252 – 259.
8. Shukla Y, Taneja P, Arora A, Sinha N, Mutagenic Potential of Mancozeb in Salmonella typhimurium, J Environmental Pathology, Toxicology and Oncology, 2004; 4(23): 60 – 65.
9. Ma TH, The role of plant systems for the detection of environmental mutagens and carcinogens. Mutat Res., 1999; 437: 97-100.
10. Sharma S, Vig AP, Genotoxicity of Atrazine, Avenoxan, Diuron and Quizalofop-p-ethyl Herbicides using the Allium cepa Root Chromosomal Aberration Assay. Terrestrial and Aquatic Environmental Toxicology, 2012; 6(2): 90-95.
11. Cabaravdic M, Induction of Chromosome Aberrations in the Allium cepa Test system caused by the exposure of cells to benzo(a)pyrene. Med Arh., 2010; 64(4): 215-218.
12. Aiub C, Mazzel JL, Ribeiro LF, Felzenszwalbi, Evaluation of nitroreductase and acetyltrans-ferase participation in N-nitrosodiethylamine genotoxicity. Chem Biol. Interact, 2006; 161: 146-154.
13. Thais C, Dania EC, Maria A, Pesticide Biochemistry and Physiology. Mechanism of micronuclei formation in Polyploidizated cells of Allium cepa exposed to trifluralin herbicides, 2007; 3(88): 252 – 259.
14. Leme DM, Marin-Morales MA, Allium cepa test in environmental monitoring: Are view on its application. Mutat Res., 2009; 682(1): 71-81.
15. Grant WF. The present status of higher plant bioassays for detection of environmental mutagens. Mutat Res., 1994; 310: 175-185.
16. Radic S, Stipanicev D, Vujcic V, Rajcic MM, Sirac S, Pevale B, The evalution of surface wastewater genetoxicity using the Allium cepa test, 2010; 408: 1228-1233.
17. Fiskesjo G, The Allium test as a standard in environmental monitoring. Hereditas, 1985; 102: 99-112.
18. Trushin MV, Ratushnyak AY, Arkharova IA, Ratushnyak AA, Genetc Al-terations Ravealed in Allium cepa-test system under the Action of some Xenobiotics. World Applied Sciences Journal, 2013; 22(3): 342-344.
19. Lijinsky W, Chemistry and Biology of N-Nitroso compound, Cambridge University Press. Cambridge, N.Y. USA. 1992; 463.
20. Cabrera GL, Rodriguez DMG, Genotoxicity of leachates from a landfill using three biossays. Mutat Res., 1999; 426: 207-210.
21. Turkoglu S. Genotoxicity of five food preservatives tested on root tips of alliun cepa L. Mutat Res., 2007; 626: 4-14.
22. Badr A, Ibrahim AG, Effect of herbicide glean on mitosis, chromosome and nucleic acids inallium cepa and vicia faba root meristems. Cytol, 1987; 52: 293-302.
23. Rank J, Nielsen MH, Genotoxicity testing of wastewater using the Allium cepa anaphasetelophase chromosome aberration assay. Mutat Res., 1998; 418: 113-9.
24. Rank J, The method of Alliumanaphase-telophase chromosome aberration assay. Ekologija, 2003; 1: 38-42.
25. Paul A, Nag S, Sinha N, Cytological effect of Blitox on root mitosis of Allium cepa L. International Journal of Scietific and Research Publication, 2013; 3: 1-3.
26. Olorunfemi DI, Ogieseri UM, Akinboro A, GenotoxicityScreening of Industrial Effuents using Onion bulbs(Allium cepaL.). Journal of Applied Science and Enviromental Management, 2011; 15(1): 211-216.
27. Amin AW. Cytotoxicity testing of sewage water treatment using Allium cepa Chromosome aberrations assay. Pakistan Journal of Biological Science, 2002; 5(2): 1884-1888.