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Valuation of Genotoxic Effect of Aqueous Organic Compound with Allium Sativum [Garlic]

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Abstract: *The current study was deliberate to estimate the genotoxic effect of 1,2,4,5-tetrazin using Allium sativum [Garlic] root chromosomal abnormality analyse. Root tips of Allium sativum were treated with different concentrations of 0.001M, 0.002M, 0.003M, 1,2,4,5-tetrazin with 10% Dimethyl Sulfoxide-Water were used in the current study. After treatment Various types of physiological hydrolyzed, squashed, mitotic index (MI), cytological abnormalities and Chromosomal aberrations rate were scored. Then the results exposed a genotoxicity effect as well as significance reduction in the Mitotic index of toxic effect was found to be dose dependent.*

Keywords: Allium Sativum., Garlic, Mitotic Index, 1,2,4,5-Tetrazin, Chromosomal Aberrations

I. INTRODUCTION

Development of industrial, technological as well as agricultural revolutions have resulted in an ever-growing negative outcome on the atmosphere in terms of its contamination and dilapidation. Various etymology effect, such as manufacturing, processing, conveyance and consumption besides reducing extra stock of ordinary properties enhance pressure to the atmosphere by accruing dissimilar dangerous constituents [1-3].In current ages, most of the use of pesticides, fertilizers, continuous air emission from industrial sources and vehicular traffic have contaminated environment with various organic chemicals [4-5]. 1,2,4,5-tetrazines have attracted much consideration due to their diverse medicinal, biology, industrial and agricultural importance. 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 applied in combating fungal and bacterial infection on plants. 1,2,4,5-tetrazines are show anticonvulsant, anti-inflammatory, 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 attempt is to assess the genotoxic effects of 1,2,4,5 – tetrazine. 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 [11-13].

Among various plant bio-assays, chromosomal aberration assay in Allium sativum rootis one of the most dependable bio-assays which can be useful to notice extensive series of genetic damages[14-16].

In this study, it was used for mitotic index and genotoxic effect in Allium sativum with ,2,4,5-tetrazin with 10% Dimethyl Sulfoxide-Water treatment each of the selected parameter shows a toxic effect on tissue from a different angle. So this work was intent to investigate the cytologic, genotoxic changes induced by 2,4,5-tetrazin with 10% Dimethyl Sulfoxide-Water effect.

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II. Material and Methods

The healthy bulbs of allium sativum were purchased from market. These bulbs were located in trivialbeaker with the basal ends dipping in distilled water at room temperature. Then the new emerged roots of 2-3 cm in length were treated with different concentrations of 0.001M, 0.002M, 0.003M, 1,2,4,5-tetrazin with 10% Dimethyl Sulfoxide-Water were used in the current study. A negative control has been treated with distilled water. After 24h, the roots of each bulb were takeout and immovable in recently prepared and cool mixture (4-10 ^{o}C) containing three parts of methanol and one part of glacial acetic acid (3:3, v/v) for 24h. Roots can be deposited in fixing used for more than a few days or weeks. Intended for working out root tip genetic material slides acetoorce in crush method has been used to analyse mitotic index, cytotoxic effects and CAs. Taken 1N hydrochloric acid (HCl) at 60^{0}C for 4-5 min for hydrolysed the root tips of bulbs of allium sativum. The cell wall has to soften by hydrolysis with acid. Then, roots are transferred to distilled water and port for some times (Min). Then roots were moved on clean slide. Root tips were used for each slide. Roots tips place on slide, tips were creased in drop of 2% acetoorcein [17] with the plane end of metallic rod (taper) and squashed under a cover slip. The pressure was applied under several thickness of blotting 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 irregularities such as anaphase bridges, laggards, micronuclei and stickiness were recorded in the mitotic cells [18].

III. Result Analysis and Discussion

Treatment of allium sativum roots with different concentration of 0.001M, 0.002M, 0.003M of 1,2,4,5-tetrazin with 10% Dimethyl Sulfoxide-Water revealed different types of chromosomal aberrations and cytological abnormalities such as mitotic index anaphase, metaphases, bridges, laggards, micronuclei and stickiness.

In different stages of mitosis, the crush preparations of root tip cells of negative control segments revealed a large number of normal isolating cells. However, after root tips treated with different concentrations of 1,2,4,5-tetrazin with 10% Dimethyl Sulfoxide-Water were squashed, a number of dividing cells with different kinds of aberrations such as c-mitosis, disturbed anaphases, stickiness, laggards, disturbed anaphases, abnormal metaphases, bridges and chromosomal breaks were observed.

According to (Saxena et al.) carbofuran, allium sativum showing to carbofuran for 24 hrsof harmfulness was disclosed that chromosomal and mitotic deviations in the root meristem cells.[19]. chromosomal disturbance were determined such as micronucleus, Hyperchromasia, later segregation, c-mitosis, chromosome loss, pulverised nucleus, chromosomal adherence, chromatin globules. According to outcomes, the total chromosomal changing improved with increasing doses of 1,2,4,5-tetrazin with 10% Dimethyl Sulfoxide-Waterand the control as compared to revelation time.[20-21].

In present study, genotoxicaltering was determined intracellular levels. At this time Outcomes attainable approve the largest sensitivity of root with considerable upsurges with cell disfigurementl ateraction of cell through doses of 24-72 hrs [22]. of increasing doses of 1,2,4,5-tetrazin with 10% Dimethyl Sulfoxide-Water. Main cellular varying was found by way of different kinds of aberrations such as c-mitosis, disturbed anaphases, stickiness, laggards, disturbed anaphases, abnormal metaphases, bridges and chromosomal breaks were observed.

sub-chromatid bridges mean indecorous folding of the chromosomal threads which have caused in combination of threads and genes become attach to each other [23-24].

In the present-day work, in entire concentrations of 1,2,4,5 – tetrazine, has been found stickiness. The highest frequency of stickiness was observed at the highest concentration of 1,2,4,5 – tetrazine. In consistent with present report, stickiness has also been observed in Allium sativum following treatment with herbicide avenoxan (Gul et al., 2006); copper sulfate (Liu et al., 2009); insecticide carbofuran (Saxena, 2010)[25-26]. Lagging chromosomes/chromosome fragments (also known as laggards) are a type of physiological aberration which arises because of failure of whole chromosome or acentric fragment of a chromosome to get attached to the spindle fiber. In

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the present study, few cells with lagging chromosomes were observed with 1,2,4,5 – tetrazine. Lagging chromosomes have been observed in root tips cells of different plant species following treatment with several chemicals including pesticides like rogor in Vicia faba (Amer and Farah, 1974); thimet in H. vulgare (Singh et al., 1977). root tip cells of Allium cepa were presented lagging chromosomes reported by (Gul et al., 2006; Saxena, 2010)[27-30].

Chromosomal interruptions and chromatin granulebonds observed in the present study constituted spectrum of clastogenic aberrations. Chromosomal breaks were observed in low frequency as compared to chromatin bridges and their frequency is maximum (0.87) at 0.8 ppm. The formation of chromatin bridges may be the result of unequal exchanges resulting in the formation of dicentric chromosomes which are pulled equally to both poles at anaphase. Chromatin bridges were observed following treatment with all the concentrations of 1,2,4,5-tetrazin with 10% Dimethyl Sulfoxide-Water.

IV. Discussion

The present study exposed a dose dependent increase in the total number of aberrations following treatment with different concentrations of 1,2,4,5-tetrazin with 10% Dimethyl Sulfoxide-Water. The observed frequency of physiological aberrations was much highest dose as compared to clastogenic aberrations highest dose. Among different kinds of physiological aberrations, c- mitosis was found to be most common. C-mitosis is detected in root tip cells when any agent (physical/chemical) prevents the assembly of spindle microtubules by dissociating disulphide bonds leading to scattering of chromosomes in the cell. The term c- mitosis was coined by Levan[31]. It is possible that 1,2,4,5-tetrazin with 10% Dimethyl Sulfoxide-Water used in the current study which induced an appreciable number of cells with c-mitosis may have acted by detaching bond in disulphide is prevented the formation of any such new-fangled bond. similar tendency of rise in the incidence of c-mitosis in root tip cells of A. sativum.

V. Conclusion

The genotoxic effect of 1,2,4,5-tetrazin using Allium sativum [Garlic] 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. Therefore, it would be significant to more contaminated effect and cytological signals at molecular level to determine the probable contaminated 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	7.9553	47	33	12	10
2	Positive control	5.5870	0	0	0	0
3	0.001M	1.27	18	19	7	3
4	0.002M	1.33	19	22	9	4
5	0.003M	1.62	25	28	10	6

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

Table 2: Mitotic aberration in root tip cells of Allium sativum (garlic) treated with 0.001M,0.002M, 0.003M of 1,2,4,5-tetrazin with 10% Dimethyl Sulfoxide-Water (DMSO)

Sr. No.	Treatment	% total Aberrant Cells	% of Aberrations					
			Precocious movement (PM)	Laggards (L)	Disturbed Prophase (DP)	Disturbed Metaphase (DM)	Disturbed Anaphase (DA)	Bridge (B)
1	Negative control	0	--	--	--	--	--	--
2	Positive control	0	--	--	--	--	--	---
3	0.001M	2.02	3.3	24.45	--	--	--	--

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4	0.002M	2.21	5.8	27.40	0.1	0.1	0.1	0.22
5	0.003M	2.33	6.2	30.33	0.2	0.3	0.2	0.33

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

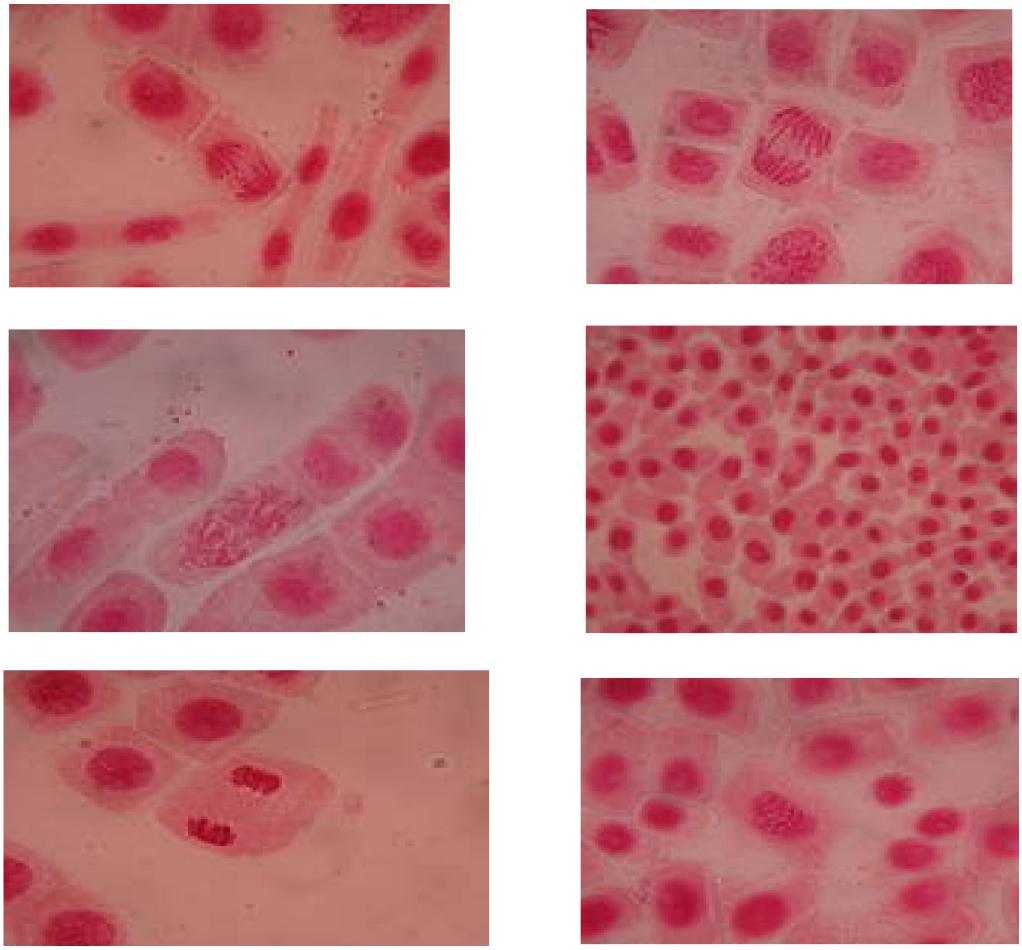

Plate I- Anaphase, Plate II-Bridge, Plate III-Disturded Metaphase,
Plate IV-laggard, Plate V-Metaphase, Plate VI-Precocious,

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