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STUDY OF CYTOGENETIC EFFECT OF PESTICIDE CHLORPYRIFOS USING CHROMOSOMAL BEHAVIOUR OF ROOT MERISTEM IN *ALLIUM CEPA* L.

KUMAR P* AND PRASAD V

Department of Botany, Patna University, Patna-800005, Bihar, India

***Corresponding Author: Mr. Pravin Kumar: E Mail: pravin32pk@gmail.com**

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ABSTRACT

The cytogenetic effects of organophosphate pesticide chloropyrifos 20% EC on root meristem of *Allium cepa* L. were studied. The bulbs with roots of *Allium cepa* L. were treated with different concentration of chlorpyriphos (0.2%, 0.4%, 0.6%, 0.8%,1.0%) for 24, 48 and 72 hours. Acetocarmine squash technique was adopted to prepare the slide of root tips of *Allium cepa* L., after getting a certain length. To measure the cytotoxic level mitotic index was prepared of microscopic observation of the cytological slide. The result indicated that Chlorpyriphos significantly increase the genetic abnormality in different concentration and treatment period when compared with control plant. Obtained data shows statistically significant difference between treated and control root meristem of *Allium cepa* L. ($P < 0.05$). All concentration and treatment period of chlorpyriphos induced a number of chromosomal aberrations in root tip cells of *Allium cepa* L. Among various chromosomal aberration, chromatin bridge, breaks, stickiness, laggard, vagrant, fragments, C-mitosis, multipolarity, ring chromosome, as well as micronuclei were observed in mitotic preparation. The RAR and PAC was also augmented. Pesticide shows that it was highly toxic to plant cell.

Keywords: Chlorpyriphos, Cytotoxicity, Mitotic Index, Chromosome abnormality, *Allium cepa* L.

INTRODUCTION

The use of pesticide has improved the agricultural productivity through inhibition of disease-causing organism, control weeds and insect pests. In spite of their agriculture benefit it is found to be affect non-target organism including human. Pesticide chlorpyriphos 20% EC commonly used in agriculture field of Bihar. The pesticide in

different concentration behaves as a genotoxic and mutagenicity and it affects the many organism which directly influence the ecosystem through food chain. This pesticide belongs to the group chlorinated organophosphate insecticide. The organophosphate are esters of phosphoric acid, thiophosphoric acid and other phosphoric acid **[1]** and are precursors of many insecticides, herbicide and nerve agent. Chlorpyrifos [diethyl o-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is one of the most used organophosphate insecticide on field crops vegetables and fruit crops. This is moderately hazardous class-II classified by WHO. Extensive use of organophosphate pesticide it creates many adverse effects on environment because of their toxicity. Pesticide enters into the food chain through dietary intake **[2]**. In man it acts on central nervous system by inhibiting acetyl cholinesterase, an enzyme that modulates the amount of neurotransmitter acetylcholine, disrupting the nerve impulse by serine phosphorylation of the hydroxyl group in the active site of the enzyme. The symptoms include loss of reflexes, headache, dizziness, nausea, convulsion coma and even death.

The *Allium cepa* test is an excellent genetic model for evaluating environmental pollutant, detecting mutagen in environments and evaluating genetic endpoints (point mutation to chromosomal alteration). Over the past decade plant test system which is alternative to mammals in testing in research and education **[3]**. The *Allium cepa* is an efficient test material for chemical screening and situ monitoring for cytogenotoxicity due to meristematic nature of plant root **[4]** and low chromosome number (2n=16) and is large in terms of structure. Onion is cheaper and easy to obtained as they can be grown any season around of the year **[5]**. for these reasons, *A. cepa* was chosen as the test material in this research. In present investigation *Allium cepa* test has been used to evaluate DNA damage in the mitotic cycle. MI is the basic criteria to analyze cytogenotoxicity for all living organism **[6]**. The ratio of mitotic index shows the potential of cytotoxicity **[7, 8]**. Cytotoxic substances show their effects on mitosis by inhibiting microtubule formation **[9]**. Present study, it was aimed to investigate the cytogenotoxicity caused by roots of *Allium cepa* L. with the help of mitosis. For this purposes, pesticide chlorpyrifos 20% EC was treated to the roots of *Allium cepa* with varying different concentration and duration. Different chromosome abnormality was seen to evaluate the mitotic index for determining cytotoxicity. During literature survey no results was obtained regarding cytotoxic effects of the pesticide chlorpyrifos 20% EC.

MATERIAL AND METHOD

Fresh healthy onion bulb of *Allium cepa* L. of approximately same size were collected from local market. The old roots were removed from the bulb and exposed root disc were suspended into plastic cups containing distilled water for three days to facilitate root growth. The onion bulbs were transferred from the plastic cups containing the distilled water to those containing the different concentration of Chlorpyrifos 20% EC (0.2%, 0.4%, 0.6%, 0.8% and 1.0% respectively) while some of the bulbs were transferred into new plastic cups containing distilled water to serve as control. The bulbs were arranged in different sets for their treatment in different pesticide at different concentration for different duration like 24, 48 and 72 hours. After completion of treatment period and getting the size of near about 1c.m. the root tips were cut at appropriate time. Appropriate time is an important factor for getting stage of cell division in different season. Root tip were collected from the control set of treatment treated with PDB (*p*-dichlorobenzene) solution for 3-4 hours. After treated root tips with PDB were subjected to repeated wash for 3 to 4 times with dd water. Similarly, the roots collected from different set from treated line of experiments were not subjected to pretreatment process as it may alter the change the result. After washed pre-treated roots kept in carnoy's fixative (Absolute alcohol, glacial acetic acid – 3:1) for 24 hours **[10]**. and washed three times with distilled water and finally kept in 70% alcohol for further investigation.

Preparation of slides and Microscopic examination

At the mean time 2% acetocarmine solution was prepared for staining the root tips after that squash technique was adopted to prepare the cytological slide **[11]**. Root tips were taken out from 70% alcohol and were hydrolyzed with 1N HCl at 60º C for 10 minutes in order dissolve cell wall and washed with distilled water thrice and stored. The root was transferred on a glass slide and cut the root tip (1-2mm) with surgical blade and then dipped in a drop of 2% acetocarmine for 2 minutes. The cover slip was carefully placed over the slide by avoiding the entry of air bubble. Finally, pressed the section of slide containing stained root tip by thumb pressure by wrapping the slide with blotting paper which help to absorbed extra stained. The edge of cover slide was sealed with clear nail varnish for preservation **[12]**.

For the determination of mitotic index (MI) and the frequencies of chromosomal aberration (CA). All prepared slides were subjected to microphotography with the help of microscope with camera attachment. Objective lenses taken into consideration were of 10X, 40X and 100 X magnification. Cider oil emulsion was applied for the lens

of 100 X magnification. Five slides were prepared from each set of treatment and, on every slide almost 1000 cells were observed.

Assessing of cytotoxicity

Cytogenotoxicity Potential was determined by Mitotic index (MI), Relative Abnormality Rate (RAR) and percentage aberrant cell (Gupta *et al*., 2020) **[13]**. The mitotic index and chromosomal aberration were calculated according to the standard method described by Bakare *et al.,* **[14]** The total number of cells of each set of treatment was calculated by this method-

$$Mitotic\ index = \frac{Number\ of\ dividing\ cell}{total\ number\ of\ cells} X\ 100$$

In this study, at least 1000 cell were counted in each slide and mitotic index was calculated by determining the cells undergoing mitosis within 1000 cells and stage of their division.

The percentage of aberrant cells can be calculated by eq-

$$\textbf{\textit{\% of aberrant cells}} = \frac{\textbf{\textit{Number of aberrant cells}}}{\textbf{\textit{Total number of cells}}} \boldsymbol{X\ 100}$$

The percentage of aberrant cells can be calculated by eq-

$$\textbf{\textit{\% of aberrant cells (PAC)}} = \frac{\textbf{\textit{Number of aberrant cells}}}{\textbf{\textit{Total number of cells}}} \boldsymbol{X\ 100}$$

$$\textbf{\textit{\% of Relative abnormality rate (RAR)}} = \frac{\textbf{\textit{Number of aberrant cells}}}{\textbf{\textit{Total number of diving cells}}} \boldsymbol{X\ 100}$$

The data was analyzed using SPSS 16.0 software with a single significance level of $p \leq 0.05$. the mitotic index (MI), and chromosomal abnormalities were compared using one-way ANOVA.

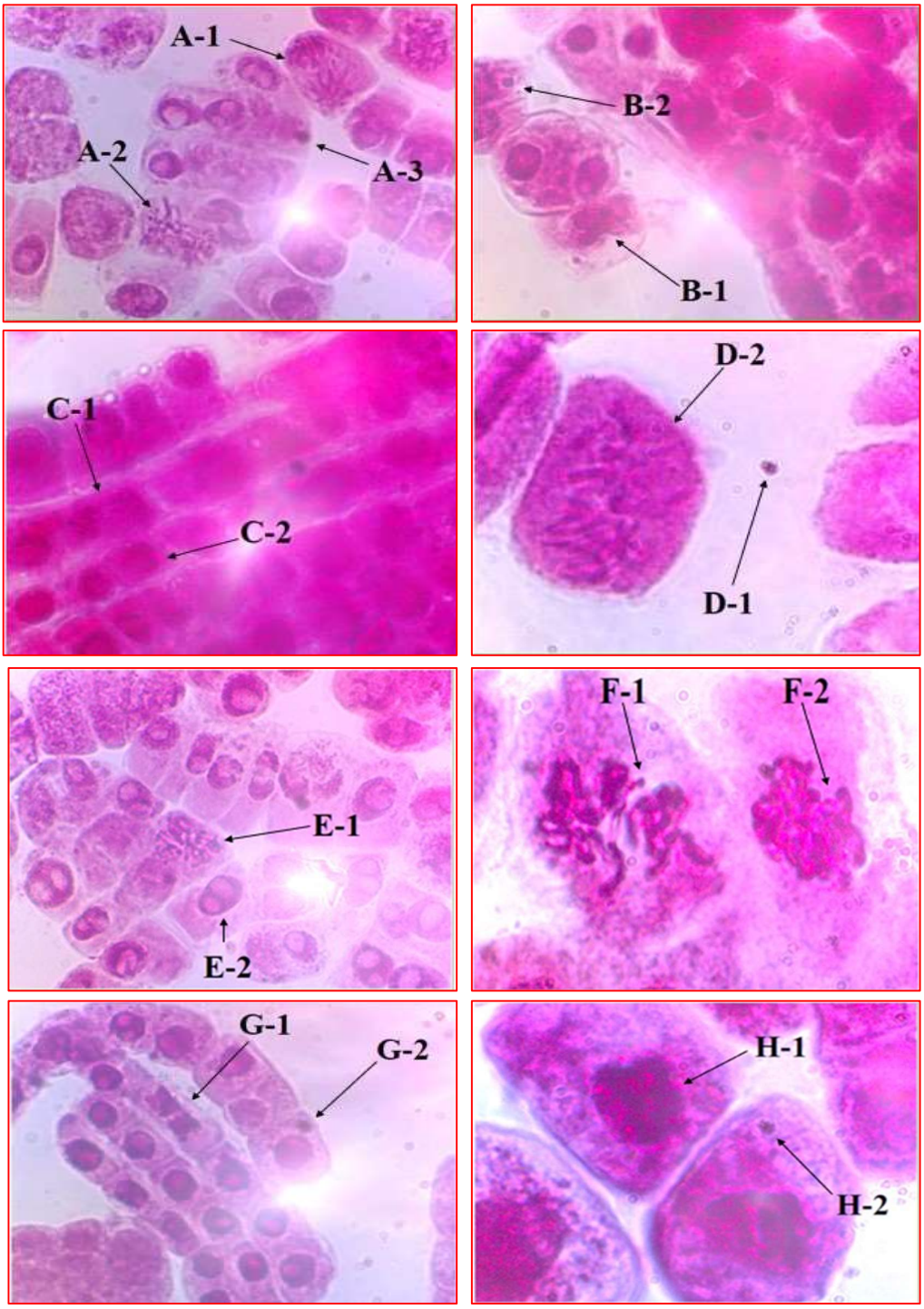

Figure 1: A1: anaphase with laggard; A2: Disoriented anaphase; A3: Micronuclei; B1: Break & fragmentation; B2: Micronuclei; C1: Disturbed anaphase; C2: C- Metaphase; D1: Micronuclei; D2: Sticky metaphase; E1: vagrant chromosome; E2: Binuclear & Nuclear lesion; F1: Stickiness; F2: Prophase; G1: Anaphase with bridge; G2: Micronuclei; H1: clumped chromosome; H2: Micronuclei

Table 1: Chromosomal aberration in root meristem of *Allium cepa* exposed to different concentration of pesticide Chlorpyrifos after 24, 48 and 72 h

Duration of treatment	Concentration of test pesticide (%)	Total no. of cell observed	Total no. of dividing cell	Total no. of chromosome abnormality	Mitotic Index (MI)	Percentage Aberrant Cell (PAC)
24h	Control	1031	160	1	15.51 ± 0.47	0.09 ± 0.05
	0.2	1031	150	2	14.54 ± 0.21	0.19 ± 0.05
	0.4	1048	130	5	12.4 ± 0.23	0.47 ± 0.16
	0.6	1048	109	11	10.4 ± 0.25	1.04 ± 0.19
	0.8	1000	85	18	8.5 ± 0.54	1.8 ± 0.21
	1.0	1011	68	23	6.72 ± 0.36	2.27 ± 0.13
48 h	Control	1028	161	1	15.66 ± 0.63	0.09 ± 0.05
	0.2	1058	148	4	13.98 ± 0.04	0.37 ± 0.16
	0.4	1081	121	7	11.19 ± 0.10	0.64 ± 0.13
	0.6	988	100	12	10.12 ± 0.42	1.21 ± 0.26
	0.8	1021	76	20	7.44 ± 0.24	1.95 ± 0.25
	1.0	998	57	24	5.71 ± 0.33	2.4 ± 0.42
72 h	Control	1032	162	1	15.69 ± 1.15	0.09 ± 0.10
	0.2	1050	140	5	13.33 ± 0.23	0.47 ± 0.14
	0.4	1030	115	9	11.16 ± 0.41	0.87 ± 0.1
	0.6	1110	92	16	8.28 ± 0.13	1.44 ± 0.03
	0.8	1042	70	21	6.71 ± 0.16	2.01 ± 0.51
	1.0	1012	52	29	5.13 ± 0.59	2.86 ± 0.64

All values are the mean of triplicates. ± S.D. (n=3), different from the control ($P \leq 0.05$)

Table 2: Various types of Chromosomal aberration in root meristem of *A. cepa* exposed to different concentration of Chlorpyrifos after 24, 48 and 72 h.

Concentration of test Pesticide	Duration of Treatments	Types of Chromosomal Aberration (CA)							Total aberrant cell
		Stickiness	C-Metaphase	Disturbed Anaphase	Clumping Anaphase	Laggard	Bridge	Micronuclei	
Control	24 h	-	1	-	-	-	-	-	1
	48 h	1	-	-	-	-	-	-	1
	72 h	-	1	-	-	-	-	-	1
0.2%	24 h	1	-	1	-	-	-	-	2
	48 h	1	2	1	-	-	-	-	4
	72 h	1	2	2	-	-	-	-	5
0.4%	24 h	3	-	1	1	-	-	-	5
	48 h	2	1	2	2	-	-	-	7
	72 h	2	2	3	1	1	-	-	9
0.6%	24 h	1	3	2	2	2	1	-	11
	48 h	2	3	1	2	2	1	1	12
	72 h	4	4	3	2	2	-	1	16
0.8%	24 h	5	5	4	3	-	1	-	18
	48 h	4	3	2	4	3	2	2	20
	72 h	4	2	3	4	3	3	2	21
1.0%	24 h	5	3	2	5	5	2	1	23
	48 h	5	2	3	4	4	3	3	24
	72 h	6	5	3	4	5	3	3	29

All values are the mean of triplicates. ± S.D. (n=3); different from the control ($P \leq 0.05$)

Table 3: Percentage of various Chromosomal (CA) Aberration and Relative Abnormality Rate (RAR) in root meristem of *A. cepa* exposed to different concentration of Chlorpyrifos after 24, 48 and 72 h.

Concentration of test Pesticide	Duration of Treatments	Types of Chromosomal Aberration (CA)							Total aberrant cell	Relative Abnormality Rate (RAR)
		Stickiness	C-Metaphase	Disturbed Anaphase	Clumping Anaphase	Laggard	Bridge	Micronuclei		
Control	24 h		100	-	-	-	-	-	1	0.62 ± 0.04
	48 h	100	-	-	-	-	-	-	1	0.62 ± 0.40
	72 h	-	100	-	-	-	-	-	1	0.61 ± 0.85
0.2%	24 h	50	-	50	-	-	-	-	2	1.33 ± 0.38
	48 h	25	50	25	-	-	-	-	4	2.7 ± 1.18
	72 h	20	40	40	-	-	-	-	5	3.57 ± 1.06
0.4%	24 h	60	-	20	20	-	-	-	5	3.84 ± 1.31
	48 h	28.57	14.28	28.57	28.57	-	-	-	7	5.78 ± 1.19
	72 h	22.22	22.22	33.33	11.11	11.11	-	-	9	7.82 ± 0.07
0.6%	24 h	9.09	27.27	18.18	18.18	18.18	9.09	-	11	10.09 ± 1.69
	48 h	16.66	25	8.33	16.66	16.66	8.33	8.33	12	12 ± 2.84
	72 h	25	25	18.75	12.5	12.5	-	6.25	16	17.39 ± 0.65
0.8%	24 h	27.77	27.77	22.22	16.66	-	5.55	-	18	21.17 ± 1.47
	48 h	20	15	10	20	15	10	10	20	26.31 ± 3.18
	72 h	19.04	9.52	14.28	19.04	14.28	14.28	9.52	21	30 ± 7.16
1.0%	24 h	21.73	13.04	8.69	21.73	21.73	8.69	4.34	23	33.82 ± 0.28
	48 h	20.83	8.33	12.5	16.66	16.66	12.5	12.5	24	42.1 ± 6.83
	72 h	20.68	17.24	10.34	13.79	17.24	10.34	10.34	29	55.76 ± 8.39

All values are the mean of triplicates. ± S.D. (n=3); different from the control ($P \leq 0.05$)

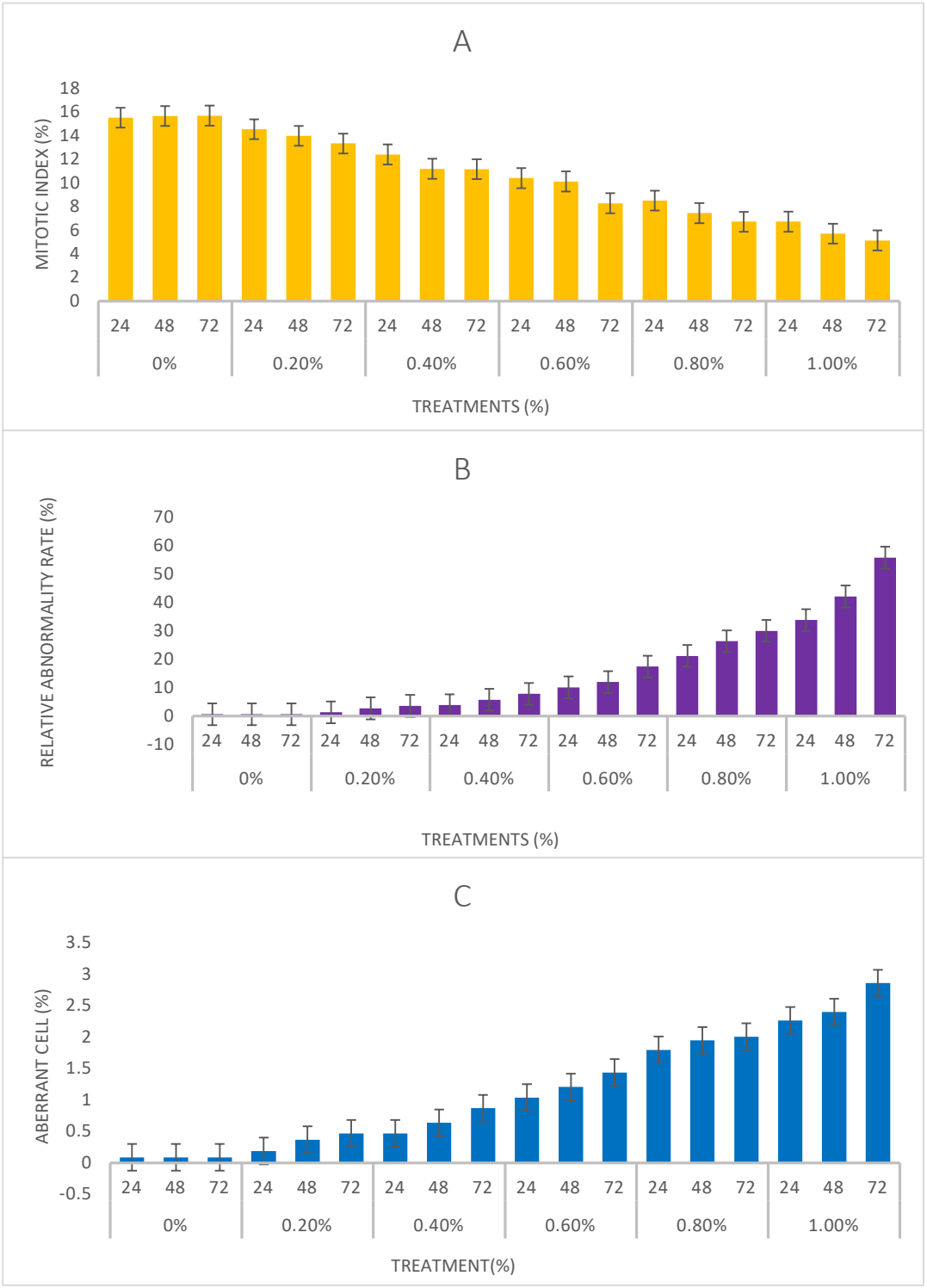

Figure 2: Effect of different concentration of Profenofos on [A] Mitotic Index; [B] Relative abnormality (%); [C] Aberrant cell (%) in *Allium cepa* L. at 24, 48 and 72 h. All values are mean of triplicates. ±S.D.

RESULTS AND DISCUSSION

It is evident from the results the mitotic index decreased considerably in the different treatment groups. Microscopic examination shows that squashes of *Allium cepa* L. root tip meristem cells shows that Chlorpyrifos treatments induced number of chromosome abnormalities when compared with control plants. The increase of mitotic abnormalities was depending on the increasing treatment period and concentration. The most common abnormalities were stickiness, c-mitosis, and disturbed metaphase. In addition, at anaphase and telophase, fragments, bridges, lagging chromosome and irregular anaphase were also observed. To evaluate the cytotogenotoxicity of the different concentration of the pesticide on mitotic index. The result obtained from this study reveal a concentration dependent decrease in the mitotic index in the cells of *Allium cepa*. This finding agreed with Sutan *et al.,* 2014 **[15]** who reported that pesticide induce a decrease in mitotic index in *Allium cepa*. Mitotic index is an acceptable measure of cytotoxicity for all living organism (Smaka-kinel *et al*., 2003) **[8]**. The cytotoxicity level can be determined by the decreased rate of mitotic index. A decrease below 50% usually has sublethal effects **[16]**. If mitotic index decreases below 22% of the control, that it causes lethal effect on test organism **[17]**. Generally cytotoxic substances inhibiting mitosis effect the microtubule configuration **[18]**. According to many investigators, abnormalities due to inhibition of spindle formation such as C-mitosis, multipolar anaphases, sticky and vagrant chromosome, reflects high toxicity of pollutants **[19-22]**. The reduction of mitotic activity may result from a blocking of G1 stage suppressing DNA synthesis **[23]**. Among them, stickiness of chromosome was frequently observed. This is due to the inhibition of spindle formation **[24]**. Improper folding of chromosome fires that make the chromatid connected by subchromatid bridges as a result of sticky chromosome **[25]**. The frequency of chromosome stickiness significantly increased ($P < 0.05$) with increasing dose and duration of treatment and maximum at high concentration of 72 hours (20.68%). It reflects highly toxic to the cell, probably leading to cell death. Micronuclei formation in root meristem cell were also observed. It is an indication of mutagenic effect of certain physical and chemical factors **[26]**. Hence, MN is single method to assess the mutagenic effect of all test chemicals. Kirsch *et al*.,2011 **[27]** Stated that formation of micronuclei due to acentric fragments or laggard chromosome that are not able to incorporate in daughter nuclei formation. The maximum frequency of formation of micronuclei at high concentration at 72

hours (10.34%). It causes genomic loss to the organism.

Present study, the cytotoxicity of pesticide (chlorpyrifos 20% EC) on root tips of *Aliium cepa* had reduced MI compared with distilled water which are indication of inhibition of cell division therefore, said to be cytotoxic to varying dose and concentration. There were decrease in MI observed among all three duration (24, 48 and 72hrs.). In 24hrs of treatment duration the MI was found to be 14.54 at lower concentration and 6.72 at higher concentration which is significantly lower than control plants. In 48 and 72hrs of treatment the MI was found to be 13.98 and 13.33 respectively at lower concentration and 5.71 and 5.13 at higher concentration which is significantly lower than control plants. These finding on root meristems cells of onion are suggestive of their mito-inhibitory property and this inhibition was more pronounced as well as dose and duration dependent.

The frequency of both RAR and PAC was induced as per increase in dose and duration of treatment. The RAR stimulated significantly in dose and duration dependent manner. It ranged from 0.62 to 55.76%. The maximum frequency of RAR was apparent at maximum concentration at 72 h. simultaneously PAC was also augmented as per treatment and exposure duration. The stimulation occurs from 0.19 to 2.86.

The genotoxic effects of pesticide on onion root tip cell were significantly observed in **Table 1**. There are increase in the percentage of total abnormality along with dose and duration. In 24hrs treatment duration, the total percentage of abnormality found to be 0.19 at lower concentration and 2.27 at higher concentration. In 48 and 72hrs of treatment duration, the total percentage of abnormality was found to be 0.37 and 0.47 respectively at lower concentration and 2.4 and 2.86 at higher concentration. These finding shows that duration and concentration dependent genotoxicity of chlorpyrifos. The results from the present study indicates that chlorpyrifos can induce cytotoxic and genotoxic effects on the meristematic cells of *Allium cepa* L. Mitotic activity decreased due to inhibition of DNA synthesis **[28]** or due to arrest in G2 phase of cell cycle **[29]**. Several other pesticides have also been reported to induce genotoxic abnormalities by affecting mitotic spindle **[30]**.

CONCLUSION

On the basis of observation made on different parameter it came to know that in most of the slides prepared from the treated root tips have various cytological abnormalities due to mutagenic effects of pesticide. The pesticide Chlorpyrifos 20% EC has a mito-depressive effect on the mitotic index, and also cause severe cytological and chromosomal aberration on

the cells in concentration dependent manner. Most of the pesticide have heavy metals which are considered as carcinogenic substances so treatment of crop plants with such pesticide is not safe for human health. The frequency of chromosomal abnormality directly associated with genotoxic agent which is human health concern. This pesticide enters into the food chain through dietary intake and threats to organism and environment. Therefore, uncontrolled use of pesticide has led to the reduced connectivity, loss of biodiversity, ecological imbalance environmental stability decreased. So that no doubt pesticide should be used in the crop plant but should not exceed in threshold dose. High concentration of pesticide inhibited the crop plant or may be death of plants.

Authors contribution statement

The first author Pravin Kumar, Ph. D. Research Scholar is responsible for sampling, testing, observation and data analysis of the work done and wrote the paper with input from Dr. Vinod Prasad, Ph. D. supervisor. Finally, both authors have discussed the results and contributed to the manuscript.

Conflict of interest

Conflict of interest declared none by authors.

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