



Cytotoxic assessment of pesticide dimethoate by using chromosomal behavior of root meristem in *Allium cepa* L.

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ABSTRACT

Dimethoate 30% EC is a potential genotoxic pesticide that has found wide usage in agriculture. Considering its impact on biota, the present study was designed to assess the cytotoxic and genotoxic effects of various concentration of pesticide by using the *Allium cepa* test. The roots were exposed to dimethoate pesticide concentration (0.2%, 0.4%, 0.6%, 0.8% and 1.0%) for the aforementioned evaluation. Root growth was determined in terms of the mitotic indexes at a time interval of 24, 48 and 72hrs. The results indicate significant ($p < 0.05$) reduction of mitotic index in dose-duration dependent manner over to control. Moreover, many cytological aberrations were also observed like cytolysis, elongated nuclei, chromosome fragmentation, sticky metaphase and anaphase, laggard chromosome, c-metaphase, and anaphase bridges. The RAR, PAC and micronuclei frequency was also augmented. These findings confirm the cytotoxic and genotoxic potential of dimethoate that that might be toxic as mitodepressive action. The carcinogenicity of this xenobiotic was also evident. So that use of higher concentration of dimethoate should be not recommended as it not only causes cytogenotoxicity but might be led to death of the plants at higher doses.

Keywords: *Allium cepa*; Dimethoate; Cytological aberration; Mitotic index; Pesticide.

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INTRODUCTION

Pesticides are use all over the world and in recent years, their use has increased because it has greatly improved agriculture yield through inhibition of disease-causing organism and by acting against pest in the field and during storage of agriculture products. Large amount of these chemicals are released into the environment and many of them affect non target organism including human health. Dimethoate has been used extensively against several pest in large number of crops [1]. The persistence of Dimethoate in soil is low. This pesticide is a broad-spectrum organophosphate insecticide with systemic, contact and stomach mode of action. Dimethoate is an acetylcholinesterase inhibitor which affects the CNS (central nervous system) as well as PNS (peripheral nervous system) causing depression. It provides strength protection against prominent piercing, sucking along with chewing pests and caterpillar pests. It also has miticidal activity. The pesticide is highly compatible as well as shows synergistic impact with many other insecticides. Their target pests are Stem borer, leaf minor shoot fly, minor, aphids, thrips, sawfly, hoppers & scale. Photodegradation of this pesticide is not occurring very prominently in soil. It is highly soluble in water and It shows very weak adsorption to soil particulates and easily dissolve in water that might leads to its leaching in soil [2]. The world Health Organization (2002) categorized Dimethoate as a moderate toxicant under "Toxicity class II" (WHO, 2002) and the United Nations ranked it in Toxic Class 6.1 (FAO, 2012). It is known to efficiently increase the sister chromatid exchange frequency in toadfish lymphocytes alongwith in vitro culture of mammalian cell, in a dose dependent manner [3].

The *Allium cepa* test is an efficient test for chemical screening and in situ monitoring for genotoxicity of environmental contaminants and has been widely used to study genotoxicity of many pesticides revealing that these compounds can induce chromosomal aberration in root meristems of *Allium cepa* [4,5]. The allium test has been widely used to study the toxicity of many substances on the mechanism of cell division and to assess whether these compounds can induce chromosomal aberrations and inhibit other cytological processes [6]. *A. cepa* is an excellent test organism; its mitotic phases are very clear, there are rare spontaneous chromosomal changes, the visibility of its chromosome is high and they have fast response to exogenous substances [7]. It is under consideration that the impacts or defects occurring in *Allium cepa*

under various xenobiotics be generalized in all biota including human beings, since all cells have same basic natural properties. Hence, it is been accepted that cytological effects of xenobiotics on mitotic cells in *Allium cepa* might be considerable in with other plants along with other biota [8] because of the potential environmental and human health impact connected with heavy use of pesticide such as Dimethoate were assessed for inhibition of cell division cytotoxicity and genotoxicity using the *Allium cepa* chromosomal aberration assay.

MATERIALS AND METHODS

A fresh healthy onion bulb of *Allium cepa* of approximately same size collected from local market. The old roots were removed from the reduced stem 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 Dimethoate (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 duration of treatment ranged from 24, 48, 72 hours respectively for each of the concentrations including the control. After completion of treatment period the root tips were cut and fixed in carnoy's fixative (Absolute alcohol, glacial acetic acid – 3:1) for 24 hrs. and washed three times with distilled water. After fixation, the root tips were hydrolyzed with 1N HCl at 60°C for 10 minutes in order dissolve cell wall [4,9] and washed with distilled water thrice and stored. The root was transferred on a glass slide and cut the root tip (1-2 mm) 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 [10].

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. 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. The mitotic index and chromosomal aberration were calculated according to the standard method described by [8,11]. The total number of cells of each set of treatment was calculated by this method-

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

The percentage of aberrant cells can be calculated by eq-

$$\% \text{ of aberrant cells (PAC)} = \frac{\text{Number of aberrant cells}}{\text{Total number of cells}} \times 100$$

$$\% \text{ of Relative abnormality rate (RAR)} = \frac{\text{Number of aberrant cells}}{\text{Total number of diving cells}} \times 100$$

RESULTS AND DISCUSSION

To evaluate the cyto-genotoxicity assay in different concentration of the pesticide the mitotic index was recorded. Mitotic index of root meristem cells in *A. cepa* was ranged from 14.53 to 4.32 which declined significantly ($p \leq 0.05$) in dose dependent manner in comparison to control. The maximum inhibition of mitotic index was registered at highest dose as well as duration. It is indicator of inhibition in root growth. This root growth is the primary target of pesticide exposure and deviation in primary as well as lateral root length and root architecture have also been reported on as stress in rice and in *A. cepa* [5]. Abnormality in mitotic cell proliferation (mitotic index) along with induced clastogenic impacts in eukaryotic cells could be an efficient biomarker to evident the adverse impacts of xenobiotics [12]. This decline in mitotic index showed the negative effect of pesticide on root growth of *A. cepa*. The mitotic index declined with dose and duration dependent manner. The result obtained from this study is well corroborated with earlier reports on *A. cepa* exposed to pesticide wherein exposure caused inhibition in mitotic index in the test plants [13]. Mitotic index is an acceptable measure of cytotoxicity for all living organism [14]. This mitotic index is considered a reliable biomarker to determine the cell proliferation and its variability indicates the cytotoxicity [4]. Mahapatra et al. (2019) suggested that mitotic index inhibition and augmented mitotic/chromosomal frequency could be associated with the decline in the level of cyclin dependent kinases and Cyclin B1 proteins.

Microscopic examination shows that squashes of *A. cepa* L. root tip meristem cells shows that dimethoate treatments induced number of mitotic and chromosome abnormalities when compared with control plants. 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 1.25 to 72.72%. 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 0.19-314%. 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. Micronuclei frequency was also induced. The aneugenic/clastogenic effects in *A. cepa* were examined through relative abnormality rate (RAR) and Chromosomal aberrations (CA). (Figure 1-2; Table 1-3)

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

Concentration of pesticide	Duration of treatment	Total no. of cell scored	Total no. of dividing cell	Types of Chromosomal Abnormality (CA)							Total abnormal cells	
				Stickiness	C-Metaphase	Disturbed Anaphase	Clumping Anaphase	Laggard	Bridge	Micronuclei		
Control	24 h	1047	159	-	1	-	-	-	-	-	-	1
	48 h	1028	157	1	-	-	-	-	-	-	-	1
	72 h	1030	160	-	1	-	-	-	-	-	-	1
0.2%	24 h	1025	149	-	1	1	-	-	-	-	-	2
	48 h	1022	141	1	2	1	-	-	-	-	-	4
	72 h	1010	147	-	3	3	-	-	-	-	-	6
0.4%	24 h	1011	124	3	-	2	3	1	-	-	-	8
	48 h	1022	115	3	2	3	1	-	-	-	-	9
	72 h	1025	107	2	2	4	1	1	-	-	-	10
0.6%	24 h	1026	102	3	3	4	1	-	1	-	-	12
	48 h	1028	95	3	3	3	2	1	--	1	1	13
	72 h	1025	85	3	4	3	2	2	1	1	1	16
0.8%	24 h	1034	79	4	4	4	3	2	1	1	-	18
	48 h	1024	71	5	3	5	3	3	2	2	-	21
	72 h	1023	61	5	5	4	4	2	2	2	2	24
1.0%	24 h	1022	55	6	5	5	3	3	2	2	2	26
	48 h	1021	50	7	6	5	4	3	2	3	2	29
	72 h	1017	44	7	6	6	5	3	3	3	2	32

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

Table 2: Percentage of various aberration in root meristems of *A. cepa* exposed to different concentration of Dimethoate after 24, 48 and 72h

Concentration of test Pesticide	Duration of treatment	Sickness	Types of Chromosomal Abnormality (CA)						Relative Abnormality Rate (RAR)
			C-Metaphase	Disturbed Anaphase	Clumping Anaphase	Laggard	Bridge	Micronuclei	
Control	24 h	-	100	-	-	-	-	-	0.62 ± 0.36
	48 h	100	-	-	-	-	-	-	0.63 ± 0.35
	72 h	-	100	-	-	-	-	-	0.62 ± 0.36
0.2%	24 h	-	50	50	-	-	-	-	1.25 ± 0.40
	48 h	25	50	25	-	-	-	-	2.83 ± 0.02
	72 h	-	50	50	-	-	-	-	4.08 ± 0.18
0.4%	24 h	37.5	-	25	37.5	-	-	-	6.45 ± 0.13
	48 h	33.33	22.22	33.33	11.11	-	-	-	7.82 ± 0.54
	72 h	20	20	40	10	10	-	-	9.34 ± 0.57
0.6%	24 h	25	25	33.33	8.33	-	8.33	-	11.76 ± 0.44
	48 h	23.07	23.07	23.07	15.38	7.69	-	7.69	13.68 ± 0.76
	72 h	18.75	25	18.75	12.25	12.5	6.25	6.25	18.82 ± 1.57
0.8%	24 h	22.22	22.22	22.22	16.66	11.11	5.55	-	22.78 ± 1.24
	48 h	23.8	14.28	23.80	14.28	14.28	9.52	-	29.57 ± 0.48
	72 h	20.83	20.83	16.66	16.66	8.33	8.33	8.33	39.34 ± 1.70
1.0%	24 h	23.07	19.23	19.23	11.53	11.53	7.69	7.69	47.27 ± 3.70
	48 h	24.13	20.68	17.24	13.79	6.89	10.34	6.89	58 ± 0.47
	72 h	21.87	18.75	18.75	15.62	9.37	9.37	6.25	72.72 ± 4.03

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

Table 3: Chromosomal aberration in root meristem of *Allium cepa* exposed to different concentration of pesticide Dimethoate 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)	% aberrant cell (PAC)
24h	Control	1047	159	1	15.18 ± 0.07	0.09 ± 0.05
	0.2	1028	157	1	14.53 ± 0.09	0.19 ± 0.05
	0.4	1030	160	1	12.26 ± 0.22	0.79 ± 0.005
	0.6	1025	149	2	9.94 ± 0.13	1.16 ± 0.05
	0.8	1022	141	4	7.64 ± 0.08	1.74 ± 0.10
	1.0	1010	147	6	5.38 ± 0.49	2.54 ± 0.05
48 h	Control	1011	124	8	15.27 ± 0.35	0.09 ± 0.05
	0.2	1022	115	9	13.79 ± 0.11	0.39 ± 0.05
	0.4	1025	107	10	11.25 ± 0.10	0.88 ± 0.05
	0.6	1026	102	12	9.24 ± 0.17	1.26 ± 0.09
	0.8	1028	95	13	6.93 ± 0.08	2.05 ± 0.04
	1.0	1025	85	16	4.89 ± 0.06	2.84 ± 0.05
72 h	Control	1034	79	18	15.53 ± 0.12	0.09 ± 0.05
	0.2	1024	71	21	14.55 ± 1.71	0.59 ± 0.06
	0.4	1023	61	24	10.43 ± 0.15	0.97 ± 0.05
	0.6	1022	55	26	8.29 ± 0.25	1.56 ± 0.11
	0.8	1021	50	29	5.96 ± 0.05	2.34 ± 0.09
	1.0	1017	44	32	4.32 ± 0.25	3.14 ± 0.05

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

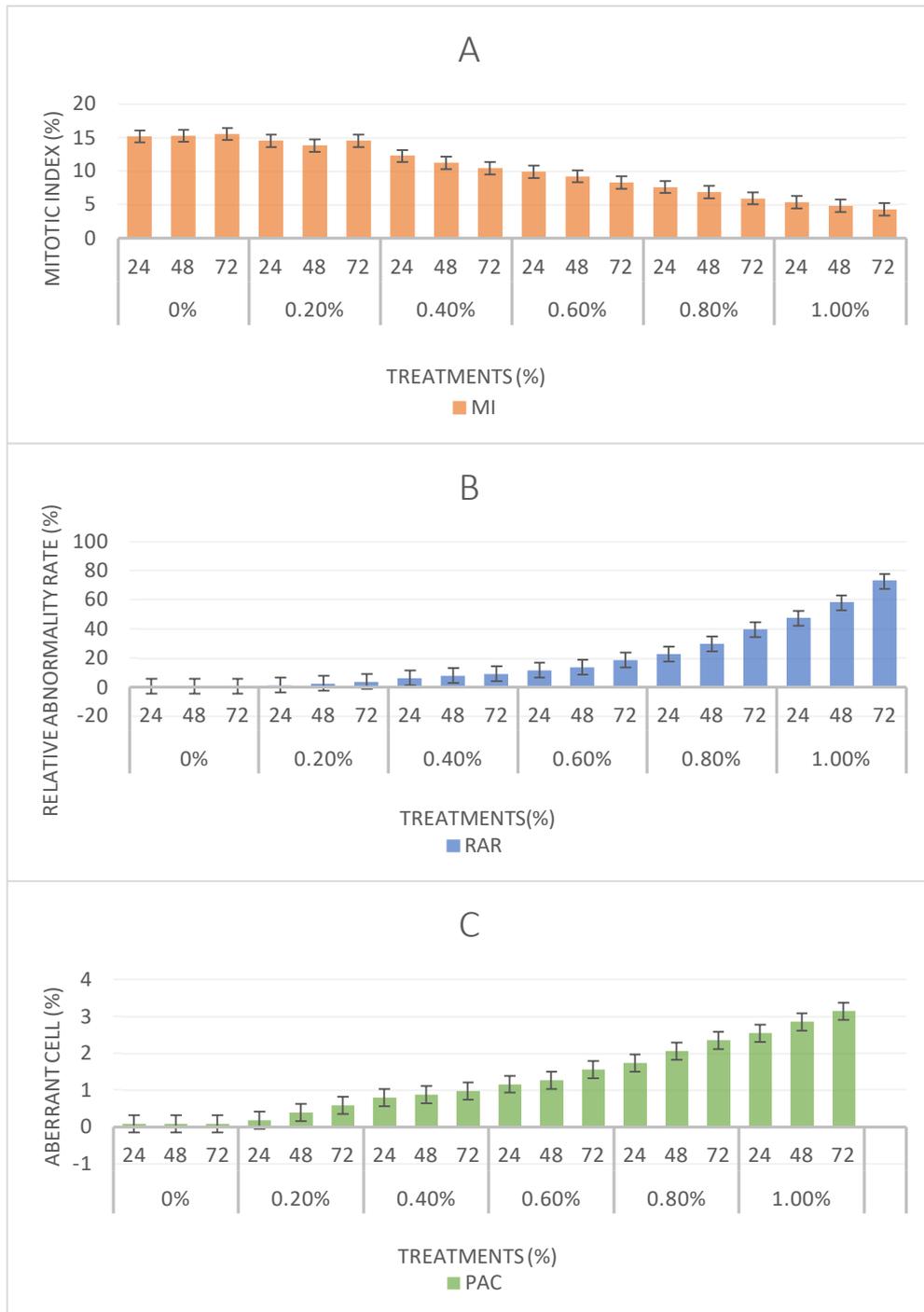


Figure 1: Effect of different concentration of Dimethoate on [A] Mitotic Index; [B] Relative abnormality (%); [C] Aberrant cell (%) in *Allium cepa* L. at 24, 48 and 72 h. All values are mean of triplicates. \pm S.D., (n=3)

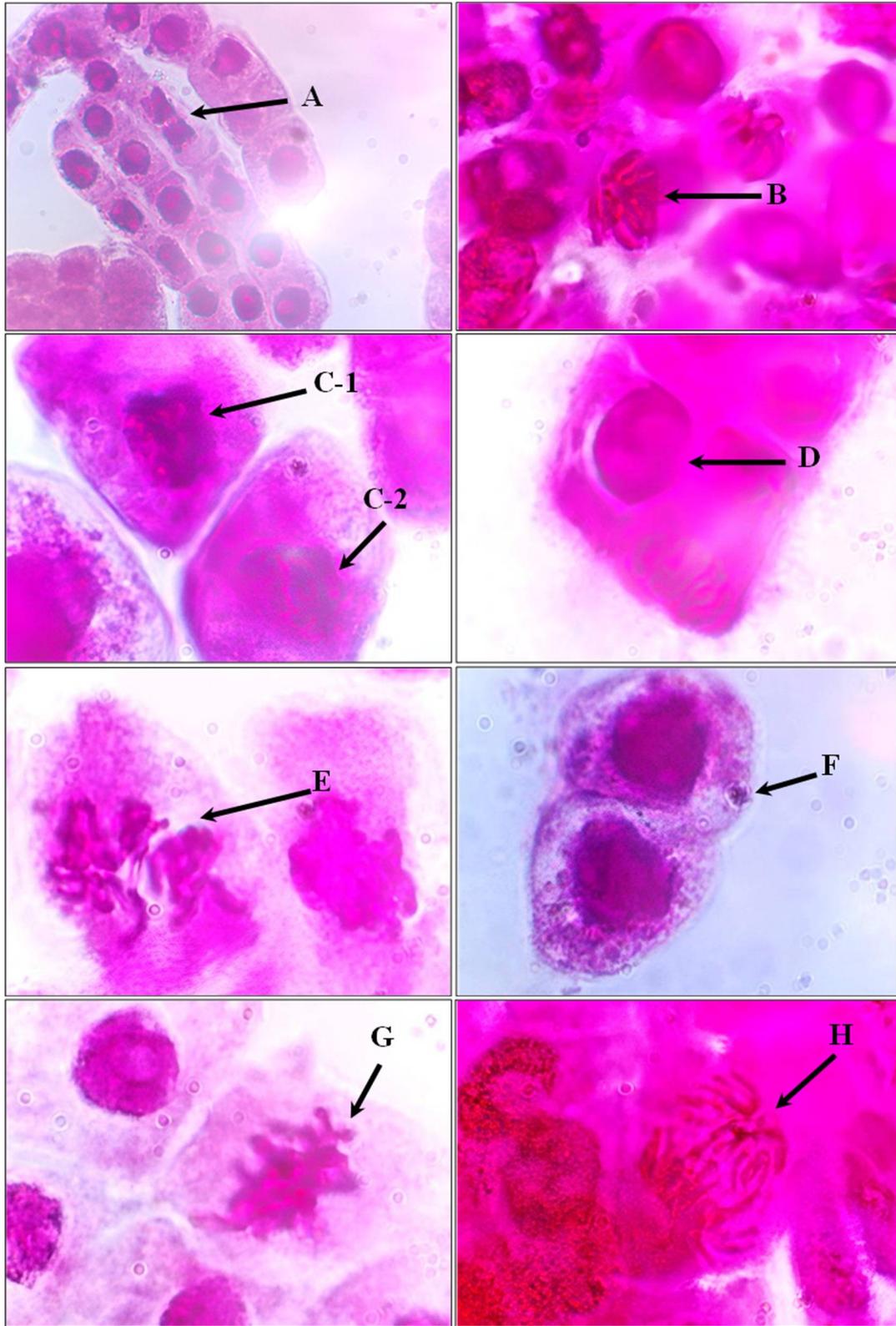


Figure 2: Various types of cytological abnormalities in root of *Allium cepa* L. (A) Sticky anaphase; (B) Disoriented anaphase; (C1) Sticky metaphase; (C2) C-metaphase; (D) Interphase cell; (E) Fragmented metaphase; (F) Micronuclei; (G) Sticky metaphase; (H) Bridge in anaphase.

Xenobiotics caused lesions in DNA result in to occurrence of chromosome abnormalities like fragments and bridges. The breakage of DNA may cause fragments or formation of bridges in anaphase and telophase through chromosome fusion. Chromosomes devoid of telomere might fuse with ends of other broken chromosomes due to being sticky to form either acentric fragments or dicentric bridges in mitosis affected by mutagens [15]. In present study, the micronuclei were increased in all treated plants. The micronuclei

are induced by acentric fragments or laggard chromosomes, which are unable to incorporate into the telophasic daughter nuclei [16]. Higher concentrations of dimethoate may have exerted a kinetic inhibition effects on the spindle apparatus leading to the abnormality in the movement of chromosomes in the cells. This mitodepressive action of the pesticides may have arisen from the disruption of normal cell metabolism due to the build-up dissolved solute leading to osmotic imbalances that resulted in clastogenic effects such as chromosome fragmentations and cytolysis observed in this study [17].

A decrease below 50% usually has sublethal effects (Panda and Sahu, 1985). If mitotic index decreases below 22% of the control, that it causes lethal effect on test organism [18]. Generally cytotoxic substances inhibiting mitosis effect the microtubule configuration [19]. 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 [20,21,22,23]. In this study, Dimethoate decrease the mitotic index at all concentration and all treatment period when compared with control plant. The decrease the mitotic index was dose dependent. At all treatment periods, the highest concentration of Dimethoate decrease mitotic activity more than other used concentration. The percentage of mitotic index decrease with the increase of cells with c-mitosis, sticky, and disturbed metaphase and anaphase since it decreases the MI in root tip cells of *A. cepa* L. Similar result were also reported in root meristem cells of *A. cepa* exposed to pendimethalin [24]. Therefore, Dimethoate can be accepted as toxic agent in this study. Chromosome stickiness characterized by chromosome clustering during any phase of cell cycle. Stickiness may be caused by genetic and environmental factor. Chromosome devoid of telomere might fused with other broken chromosome due to being sticky to form either acentric fragments or dicentric bridges in mitosis affected by mutagen [15]. Several agents have been reported to cause chromosome stickiness [25,26]. Gaullden (1987) postulated that sticky chromosome results from the defective functioning of one or two types of specific non-histone protein involving chromosome organization which are needed for chromatid separation and segregation. The altered functioning of these proteins is caused by mutation in the structural gene coding for them or by the direct action of mutagen [27]. C- mitosis is one of the consequences of inactivation of spindle apparatus connected with delay in the division of centromere [28]. Disturbed metaphase and anaphase may be due to disturbance of spindle apparatus which allow that the chromosome to spread irregularly over the cell [20].

Present study, occurrence of C-mitosis, stickiness and disturbed metaphase in root cells of *Allium cepa* L. clearly shows that accumulated effects of Dimethoate on the spindle formation. In addition to mitotic abnormalities; bridges, laggard and fragments were also observed at all treatment groups.

CONCLUSION

The results from study have shown that the Dimethoate 30% EC has a mitodepressive effects on the mitotic index, and also cause severe cytological and chromosomal aberration on the cells in concentration dependent manner. This suggests that high concentration of pesticide may lead to structural and numerical change in chromosome and it is also possible that it may cause point mutation or gene mutation in the crop plant. Any mutation may lead to the change in their chemical composition and it may also cause speciation which is harmful for the biodiversity. Change in chemical content in the crop plant due to mutation is matter of health concern. Therefore, no doubt pesticide should be used in the crop plant but it doses should not exceed the threshold dose. In other words, uncontrolled use of pesticide inhibited in the crop plant.

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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