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Cytotoxic and Mitodepressive Effect of Insecticide Profenofos on Root Meristem of *Allium cepa* L.

Pravin Kumar, Vinod Prasad

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ABSTRACT

A study was performed for cytotoxic and mitodepressive effect of pesticide through root meristem of *Allium cepa* L. Various concentration (0.2%, 0.4%, 0.6%, 0.8% and 1.0%) of organophosphate insecticide profenofos for 24, 48 and 72 hrs time points exposed to *A. cepa*. The cytotoxic and genotoxic effects were evident through inhibited mitotic division and increased percentage of chromosome abnormality. Dose and duration dependent statistically significant reduction in mitotic index was recorded. The minimum mitotic index (3.94%) was estimated at 1.0% for 72 hrs. Cytogenetic endpoints like mitotic depression, relative abnormality rate and frequency of chromosomal abnormality were found to be increased when compared to control plants. Various chromosomal aberration like stickiness, laggard, bridge, fragmentation and micronuclei were also observed. Cytogenetic biomarker is very efficient and non-expensive tool to screening and biomonitoring cytotoxicity at chromosomal and DNA level.

Pravin Kumar[1]*, Vinod Prasad[2]

[1]PhD Scholar, [2]Associate Professor
Department of Botany, Patna University, Patna 800005, Bihar, India

Email: pravin32pk@gmail.com
*Corresponding author

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INTRODUCTION

Insecticides are most commonly used against insect of agricultural crops in many countries of the world to avoid huge loss. India, one of the world largest agricultural economics, is not an exception to it. The farmers use different types of pesticide to control various diseases and pest of crops. However, most of the applied pesticide get dispersed in the environment and affects non-target organism including human health. When profenofos is applied in the agricultural field its residue spread into the environment water, soil and air and its residue in soil pose adverse impact on succeeding crops and groundwater (Bedi *et al.* 2015). Pesticides toxicity depends not only its use but also on its half life period because it determines the whether or not going to be accumulated in nature (Hanson *et al.* 2015). Profenofos toxicity causes loss of soil microflora, developmental and reproductive toxicity, multiorgan dysfunction and genotoxic to non-target organism.

The *Allium cepa* is an efficient test material for chemical screening and situ monitoring for cytogenotoxicity due to meristematic nature of plant root and low chromosome number (2n=16) and is large in terms of structure. Onion are inexpensive and easy to obtained as they can be grown any season around the year for these reason, *A. cepa* was chosen as the test material in this research. It is also known as fundamental biomarker to evaluate environmen-

tal pollution. The plant-based bioassay has been considered as simple, rapid and inexpensive tool in comparison to other standard method (Firebase and Amon 2014). Among various bioassay systems, *Allium cepa* assay is one of the most promising test systems for cyto-genotoxicity evaluation (Gupta *et al.* 2018, Mercado and Caleno 2020).

Profenofos is an organophosphate insecticide. The organophosphate are esters of phosphoric acid, thiophosphoric acid and other phosphoric acid and are precursors of many insecticides, herbicide and nerve agent. International organization for standardization (ISO) name for Profenofos (O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate) (CAS No. 41198-08-7) is one of the most used organophosphate insecticide on field crops vegetables and fruit crops. This is moderately hazardous (Toxicity Class II, WHO 2009).

MATERIALS AND METHODS

A fresh and healthy onion bulb of approximately equal size were collected from local market. The bulb was thoroughly washed with water. The loose outer scales of bulb and old roots were removed with the help of forceps so as to exposed root primordia. A series of onion bulbs were placed on plastic cup containing test pesticide (Profenofos 50% EC) of different concentration (0.2%, 0.4%, 0.6%, 0.8% and 1.0%) exposed for 24, 48 and 72 hrs at room temperature while some of the bulbs were transferred to plastic cup containing distilled water to serve as control for same treatment period. After treatment, bulbs were washed with double distilled water. The root tips of onion bulbs of each set of treatment were plucked and fixed in carnoy's fixative for 24 hrs and then transferred to 70% alcohol and stored in refrigerator for further use. After fixation the root tips were hydrolyzed with 1N HCl at 60ºC for 10 min in order to dissolve cell wall. The root was transferred on glass slide and cut the root tip (1-2 mm) with surgical blade and stained with 2% acetocarmine in 45% glacial acetic acid (v/v) followed by mordanting with iron needle to visualize the mitotic stages under microscope.

Assessment of cytotoxicity

This mitotic index is considered a reliable biomarker to determine the cell proliferation and its variability indicates the cytotoxicity (Ozkara *et al.* 2015, Gupta *et al.* 2018).

Cytogenotoxicity potential was determined by Mitotic index (MI), Relative abnormality rate (RAR) and percentage aberrant cell (Gupta *et al.* 2020).

$$\text{Mitotic index (MI)} = \frac{\text{Number of dividing cell}}{\text{Total number of cells}} \times 100$$

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.

$$\text{Mitotic depression} = \frac{\text{MI (Control)-MI (Treated)}}{\text{MI (Control)}} \times 100$$

The percentage of aberrant cells can be calculated by eq-

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

The percentage of aberrant cells can be calculated by eq-

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

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

All the experiments were repeated three times. The data shown represents the mean ± SD. The data were statistically analyzed with one-way analysis of variance (ANOVA) with significant difference ($p \leq 0.05$).

RESULTS AND DISCUSSION

Cytogenotoxic biomarker

Mitotic index and mitotic depression of root meristem cells of Allium cepa L.

Effects of profenofos 50% EC on root meristem of *Allium cepa* were studied. Dose and duration depen-

Table 1. Effect of different concentration of pesticide Profenofos 50% EC on phase index (Prophase, Metaphase, Anaphase and Telophase) with mitotic index in root meristem of *Allium cepa* L. exposed for 24, 48 and 72 hrs. All values are the mean of triplicates ±SD (n=3).

Duration of treatment	Concentration of test pesticide	No. of dividing cell	Total no. cell in prophase	Prophase index	Total no. cell in metaphase	Metaphase index	Total no. cell in anaphase	Anaphase index	Total no. cell in telophase	Telophase index	Mitotic index
24 h	0	160	79	49.37	37	23.12	26	32.91	18	11.2	15.28 ± 0.09
	0.2	136	60	44.11	30	22.05	30	22.05	16	11.76	13.2 ± 0.18
	0.4	115	49	42.6	23	20	21	18.26	22	19.13	11.18 ± 0.28
	0.6	85	35	41.17	14	16.47	15	17.64	21	24.7	8.34 ± 0.81
	0.8	65	26	40	9	13.84	10	15.38	20	30.76	6.16 ± 0.06
	1	48	18	37.5	5	10.41	6	12.5	19	39.48	4.84 ± 0.13
48 h	0	158	70	44.3	36	22.78	34	21.51	18	11.39	15.11 ± 0.43
	0.2	130	58	43.84	27	20.76	26	20	19	14.61	12.26 ± 0.23
	0.4	107	45	42.05	21	17.75	19	17.75	22	20.56	10.49 ± 0.58
	0.6	78	32	41.02	12	15.38	13	16.66	21	26.92	7.61 ± 0.34
	0.8	61	24	39.34	8	13.11	9	14.75	20	32.78	5.92 ± 0.12
	1	45	16	35.55	4	8.88	5	11.11	18	40	4.45 ± 0.12
72 h	0	150	65	43.33	36	24	32	21.33	17	11.33	14.17 ± 0.27
	0.2	121	53	43.8	25	20.66	23	19	20	16.52	11.55 ± 0.11
	0.4	90	37	41.11	15	16.66	16	17.77	22	24.44	8.68 ± 0.49
	0.6	70	28	40	10	14.28	11	15.71	21	30	6.91 ± 0.13
	0.8	55	21	38.18	7	12.72	8	14.54	19	34.54	5.23 ± 0.27
	1	40	14	35	3	7.5	4	10	19	47.5	3.94 ± 0.10

dent statistically significant reduction ($p \leq 0.05$) in mitotic index was recorded. The cell proliferation index or mitotic index includes various sub phases which also influence to toxicity. Prophase index declined from dose and duration dependent manner. It inhibited in the range of 44.11 to 37.5% at 24 hrs, 43.84 to 35.55% at 48 hrs and 43.8 to 35% at 72 hrs in different applied concentration. Similarly, metaphase index also decreased from high concentration to low concentration when exposed to 24, 48 and 72 hrs. The minimum metaphase index was recorded 7.5 at 1.0% concentration at 72 hrs. The anaphase index also followed the same trend and decreased in all test system (Table 1). Unlike to these indices, telophase index increased form lower concentration to higher concentration in applied treatment period. The stimulation in telophase index from 11.76 to 39.48% at 24 hrs, 14.61 to 40% at 48 hrs and 16.52 to 47.5% at 72 hrs respectively (Fig. 1). This variability in various phase indices is ascribe to response of root

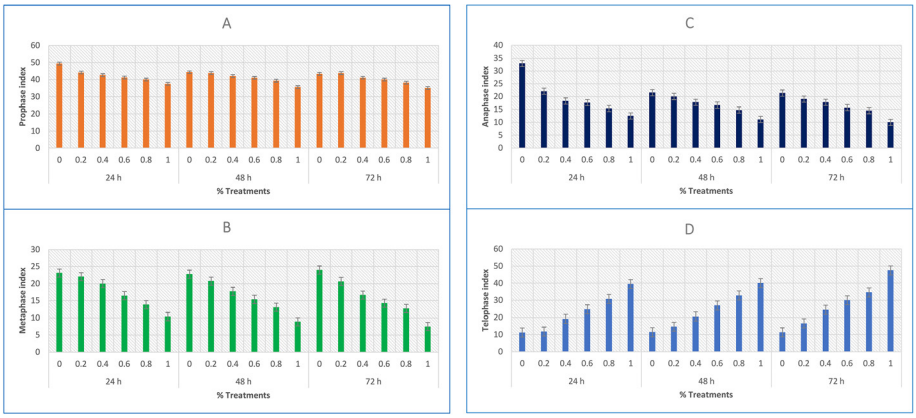

Fig. 1. Effect of different concentration (%) and period of treatment of profenofos on phase index (Prophase, Metaphase, Anaphase, Telophase) in *Allium cepa* L. at 24, 48 and 72 hrs.

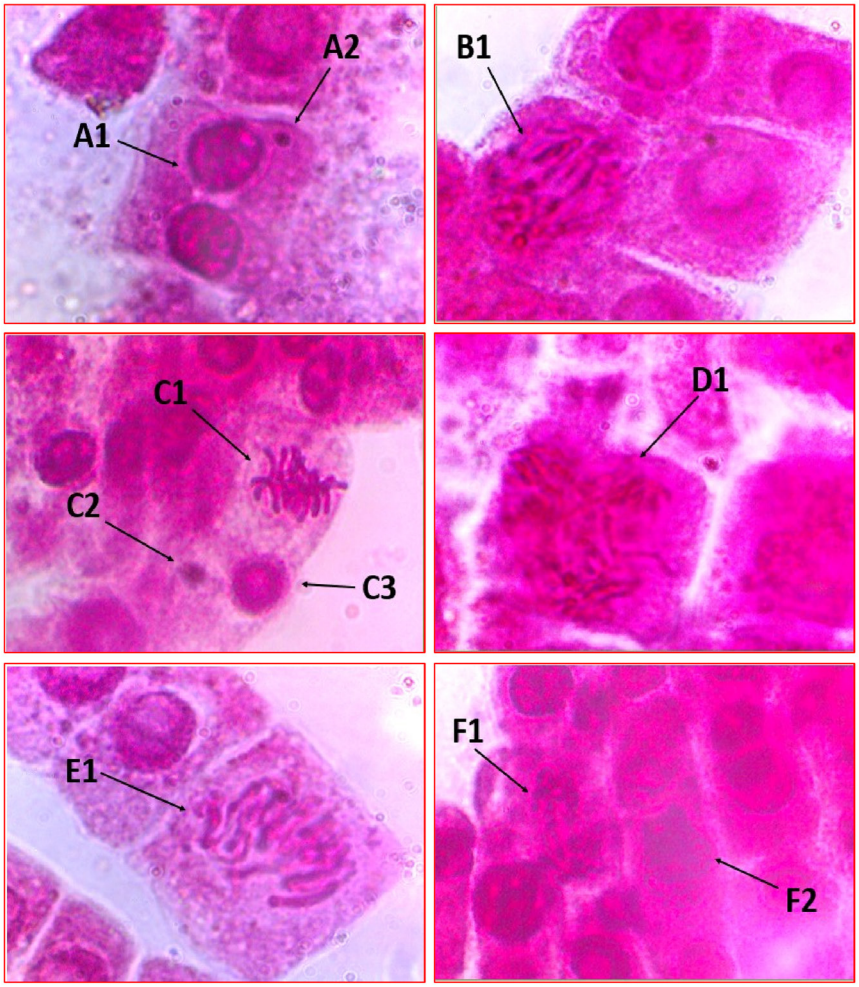

Fig. 2. A1: Telophase, A2: Micronucleus, B1: Anaphase with laggard, C1: Sticky metaphase, C2: Micronucleus, C3: Nuclear lesion, D1: Disoriented anaphase, E1: Stickiness, F1: Anaphase with bridge, F2: Clumped chromosome.

meristematic cells towards insecticide profenofos.

The mitotic index is considered a well-founded cytogenotoxic biomarker to determine the potential of cytotoxicity (Ozkara *et al.* 2015). In all set of treatments, it decreased in dose and duration dependent manner. The minimum mitotic index of 4.84%, 4.45% and 3.94% at highest concentration 1.0% in 24, 48 and 72 hrs were recorded which is lower than control plants. Reduction of mitotic activity increased with enhanced treatment duration. Significant reduction of mitotic index noted in the present study may be due to the inhibition of DNA synthesis at the S-phase or the blocking in the G_2 phase of cell cycle (Sharma and Vig 2012, Gupta *et al*. 2018).

Mitotic depression significantly increased in all examined plants when compared to the respective control value. It increased from lowest dose and treatment period to high dose and treatment period. The minimum MD was recorded (13.61%) at 0.2% concentration and 24 hrs treatment duration and maximum value (72.19%) at 1.0% and 72 hrs. Mitotic depression is inversely proportional to mitotic index (Gupta *et al.* 2018, 2020a). The mitodepressive action occurs due to inhibition in DNA synthesis or obstruction in G_1 and G_2 phase of cell cycle and this causes deviation in normal cellular proliferation (Sharma

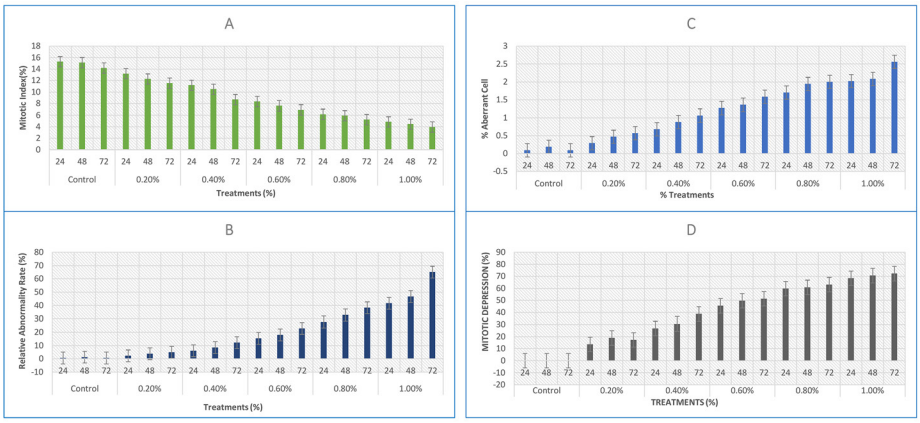

Fig. 3. Effect of different dose and duration (hours) Profenofos on (A) Mitotic index, (B) Relative abnormality (%), (C) Abnormality (%), (D) Mitotic depression in *Allium cepa* L. at 24, 48 and 72 hrs. All values are mean of triplicates±SD.

and Vig 2012, Gupta *et al*. 2018)

Relative abnormality rate and chromosomal aberration in root meristem cells of Allium cepa L.

Different chromosomal abnormalities like C-metaphase, chromosomal stickiness, laggard in anaphase, bridge and break, telophase in dividing cell as well as micronuclei in interphase cell were recorded in our investigation (Fig. 2). The aneugenic and clastogenic effects of pesticide profenofos 50% EC were indicated through Relative Abnormality Rate (RAR). RAR or PAC was observed directly proportional to concentration applied. It significantly enhanced in all test concentration in plants. RAR increased gradually from 2.2 to 41.66% at 24 hrs, 3.84 to 46.66% at 48 hrs and 4.95 to 65.0% at 72 hrs in all test concentration. (Table 2).

Table 2. Percentage of various chromosomal (CA) aberration and relative abnormality rate (RAR) in root meristem of *A. cepa* exposed to different concentration of profenofos after 24, 48 and 72 hrs. All values are the mean of triplicates ± SD (n=3).

		Types of chromosomal aberration (CA)							
Concentration of test pesticide	Duration of treatments	Stickiness	C-Metaphase	Disturbed anaphase	Clumping anaphase	Laggard	Bridge	Micronuclei	Relative abnormality rate (RAR)
Control	24 h	-	100	-	-	-	-	-	0.62 ± 0.36
	48 h	50	50	-	-	-	-	-	1.26 ± 0.68
	72 h	-	100	-	-	-	-	-	0.66 ± 0.64
0.2%	24 h	33.33	66.66	-	-	-	-	-	2.2 ± 0.43
	48 h	40	20	40	-	-	-	-	3.84 ± 0.53
	72 h	16.66	16.66	50	16.66	-	-	-	4.95 ± 0.44
0.4%	24 h	28.57	42.85	14.28	14.28	-	-	-	6.08 ± 1.23
	48 h	33.33	22.22	22.22	-	22.22	-	-	8.41 ± 1.89
	72 h	27.27	27.27	27.27	9.09	9.09	-	-	12.22 ± 3.06
0.6%	24 h	30.76	23.07	30.76	-	7.69	-	7.69	15.29 ± 2.73
	48 h	21.42	28.57	28.57	7.14	-	7.14	7.14	17.94 ± 3.08
	72 h	5	4	3	2	1	-	1	22.85 ± 4.35
0.8%	24 h	22.22	27.77	16.66	11.11	5.55	5.55	11.11	27.69 ± 5.54
	48 h	20	20	20	15	5	10	10	32.78 ± 2.94
	72 h	23.80	14.28	14.28	23.80	14.28	9.52	-	38.18 ± 8.36
1.0%	24 h	25	20	20	15	-	5	15	41.66 ± 3.44
	48 h	28.57	23.80	19.04	9.52	4.76	4.76	9.52	46.66 ± 2.15
	72 h	23.07	19.23	15.38	11.53	11.53	7.69	11.53	65 ± 18.5

Similar to RAR, MD Chromosomal aberration (CA) a potent cytogenetic biomarker was significantly enhanced in dose and duration dependent manner. Percentage aberrant cell increased from 0.29 to 2.02% at 24 hrs, 0.47 to 2.08% at 48 hrs and 0.57 to 2.56% at 72 hrs in all test concentration applied (Fig. 3). Chromosome abnormality includes changes either in chromosome structure or chromosome in number. Change in chromosome structure due to breaks of DNA, DNA synthesis inhibition, error in DNA replication. Aneuploidy and polyploidy are numerical CA caused by abnormal chromosomal segregation. In the absence of telomere, chromosome becomes sticky in nature which may join to other fragmented chromosomal ends (Nefic *et al.* 2013) and formation of either acentric fragments or dicentric bridge due to adverse impact of metals (Silveria *et al.* 2017). Frequency of stickiness irregularly increased and 23.07% in highest dose and period of treatment (1.0%, 72 hrs). Verma and Srivastava (2018) also reported stickiness in *Allium cepa* exposed to pesticide pendimethalin. Stickiness is irreversible abnormality which is caused by intense toxic effect of mutagen on chromosome that might lead to cell death also. Fatma *et al*. (2018) depicted that laggard chromosome due to weak c-mitosis that might influenced by aneuploidy. Bridge mainly developed because non-disjunction of sticky chromosome or breakage and reunion during separation at anaphase and telophase (Gupta *et al.* 2021). Bridge and break have also been recorded previously in *A. cepa* exposed to chromium and Arsenic (Gupta *et al.* 2012, 2018).

Micronuclei

In addition to the anomalies discussed above, which are symptoms of genotoxic effects of pesticide profenofos, micronuclei were also found. It is stimulated at 0.6% concentration and 24 hrs duration of treatment and reached maximum (11.53%) at highest concentration (1.0%) and treatment period (72 hrs) The micronuclei were formed due to acentric fragments or laggard chromosome caused by misrepair DNA breaks that do not get incorporated in telophasic daughter nuclei (Krisch *et al.* 2011). Micronucleus and chromosomal aberration assay in the root tips of *Allium cepa* L. have been assessed extensively in recent years for detection of potential of DNA damaging properties of environmental contaminants (Silveira *et al.* 2017).

CONCLUSION

In this study, toxicity potential of profenofos 50% EC were assessed through cytogenetic biomarker by utilizing *Allium cepa* test system. Profenofos causes threat to biota via imposing cyto-genotoxic anomalies in cell cycle along with micronuclei. Percentage of chromosomal abnormalities significantly increased in dose and duration dependent manner when compared with control plants. Pesticide showed mitodepressive effects even in lower concentration for this reason, authors recommended that high dose of pesticide should be avoided. Plants with such abnormalities may transfer the altered genetic makeup not only to the offspring but also to humans through dietary intake.

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