



Assessment of Mito-inhibitory and Genotoxic effects of two Organophosphate Pesticides in the root tip cells of *Allium cepa* L.

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Abstract: The present paper reports the effect of two organophosphate pesticides viz. Monocrotophos (MCP) and Chlorpyrifos (CPF) on mitotic index (MI) and chromosomal abnormalities in the meristematic region of root tip cells of *Allium cepa* L. Root tips of *Allium cepa* L were treated with two agrochemicals Viz Monocrotophos (MCP) and Chlorpyrifos (CPF) in different concentrations (0.12%, 0.25%, 0.50%, 0.75% and 1.00%) and were squashed in 2% acetocarmine. Mitotic index was calculated from each treatment as the number of dividing cells/100 cells and chromosomal abnormalities were counted in the different mitotic stages. The result showed that chromosomal abnormalities such as laggards, stickiness, bridges, binucleate, micronuclei and fragments gradually increased with increasing concentration of the pesticides, however mitotic index (MI) was greatly reduced by the pesticides. Interestingly, frequency of chromosomal abnormalities was recorded as Stickiness > Fragment > Bridge > Laggards > Binucleate > Micronuclei. It was further noted that the frequency of cell division was suppressed due to the toxic effect of organophosphate pesticides. The chromosomal abnormalities may be the result of direct attack of pesticides on chromosomal material. It was interesting to note that the Monocrotophos (MCP) was more effective than Chlorpyrifos (CPF).

Key Words: Organophosphate Pesticides, Mitotic index, Chromosomal abnormalities, *Allium cepa* L.

Introduction

Genotoxicity and mutagenotoxicity of pesticides for non-target organisms and their influence on ecosystems are of worldwide concern (Pimental *et al.*, 1998). Pesticides have been used to control weeds, insects and fungi in a wide range of application. The mutagenic and or carcinogenic potentials to the non-target organism have been demonstrated by several workers Badar (1983), Ahmad and Yashmin (1992), Kumar and Kumar (2000), Asita and Makhalemela (2008). In order to enhance the agricultural yields, several million tons of organic and inorganic chemicals with anti microbial and insecticidal properties are added annually to soil and environment. Some of them killing the harmful organisms not only upset the ecosystem but also produce undesirable change in higher organisms.

The mitodepressive and chromotoxic activities of pesticides in crop plants were earlier reported (Wuu and Grant, 1967; Badar, 1983). Grant (1978) pointed out that plant chromosomes are sensitive indicators to environmental pollution and suggested that the higher plant system appears to be an excellent indicators of the cytotoxic / genotoxic / mutagenic effects of

environmental mutagens therefore the plant system must be accepted as a first tier assay for detection of the possible genetic damage resulting from the use of environmental chemical.

The *Allium cepa* L. is an efficient test material for chemical screening and *insitu* monitoring for genotoxicity of environmental contaminants and has been widely used to study genotoxicity of many pesticides revealing that these compounds can induce chromosomal aberrations in root meristems of *A. cepa* L. (Thais *et al.*, 2007). Grover and Kaur (1999), Patra and Sharma (2002) were reported that *Allium cepa* has relatively large monocentric chromosomes in reduced numbers and are accepted as suitable test organism for the study of environmental mutagenesis. *Allium cepa* L. is now recommended as a stranded for environmental monitoring (Leme *et al.*, 2009)

The present investigation has been carried out to evaluate the mito-inhibitory and possible genotoxic changes induced by organophosphate pesticides (Monocrotophos and Chlorpyrifos) in the root tip cells of *Allium cepa* L.

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Material and Methods

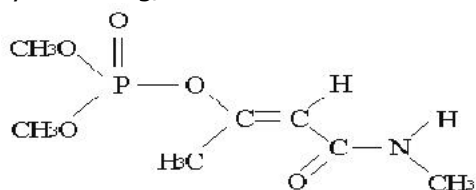
In the present investigation, *Allium cepa* L. has been used as test material of which the root tips have been treated with of two organophosphate pesticides (Monocrotophos and Chlorpyrifos) for 4hrs. The treated root tips of varying concentrations of organophosphate pesticides were squashed to assess chromotoxicity by detecting chromosomal abnormalities.

Organophosphate Pesticides

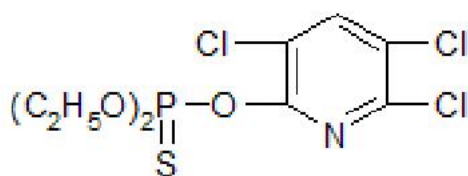
Organophosphorus compounds are usually esters, amides or thiol derivatives of phosphoric acids. They form a large family of 50,000 chemical agents with biological properties that have important and sometimes unique implications for man (Kamanyire and Karalliedde, 2008)

Monocrotophos: Monocrotophos is an organophosphate insecticide and it is acutely toxic to birds and humans its molecular formula is $C_7H_{14}NO_5P$ and Molar mass is 223.2g/mol, its melting point is approximately 55°C, boiling point is 120 ° and its density is 1.33g/cm³.

Chlorpyrifos: Chlorpyrifos is a white or colorless crystalline organophosphate insecticide having slightly skunky odor, like rotten eggs or garlic. Its molar mass is 350.59g/mol, molecular formula is $C_9H_{11}Cl_3NO_3PS$, melting point is 42°C and its density is 1.40 g/cm³.



Monocrotophos



Chlorpyrifos

Preparation of Organophosphate Pesticides solution: The solutions of varying concentrations *i.e.* 0.12%, 0.25%, 0.50%, 0.75% and 1.00%, were prepared by dissolving two organophosphate pesticides in desired amount of distilled water.

Squash preparation:

Actively growing root tips of *Allium cepa* L. were obtained from bulbs of equal size and age placed on the mouth of flask filled with distilled water. The distilled water was changed and the bulbs were placed again in beakers containing different concentrations of organophosphate pesticides for 4hrs. The control was kept undisturbed and untreated. The root tips were fixed in freshly prepared 1:3 acetobutanol for 24hrs and squash in 2% acetocarmine to study the frequency of cell division and chromosomal abnormalities in different treatments.

The Mitotic index (MI) was calculated by number of dividing cells / total number of cells scored x 100 while percentage of chromosomal aberrations by number of abnormal cells / total number of dividing cells x 100.

Results

In *Allium cepa* L. the somatic number of chromosome was noted to be $2n=16$. The spectrum of abnormalities induced by two organophosphate pesticides *viz.* Monocrotophos (MCP) and Chlorpyrifos (CPF) in the meristamatic cells of *Allium cepa* L. has been shown in table 1 and 2. It induced different types of chromosomal abnormalities such as Stickiness, fragment, diagonal metaphase/ anaphase/ telophase, bridge, laggards, micronuclei and binucleate in the different treatments. In control root tip of *Allium cepa* L. abnormalities were rare with 0.40%. Due to the treatment of organophosphate pesticides, the percentage of chromosomal abnormalities gradually increased with increasing concentration of the pesticides ranging between 8.26 to 42.00%. Both Monocrotophos (MCP) and Chlorpyrifos (CPF) treated cells had reduced Mitotic index (MI) compared with cells treated with distilled water. The depression of mitotic index was gradually increased with the increasing concentrations of the pesticides *viz.* 15.57 ± 0.66 to 04.85 ± 0.91 %. It was interesting to note that Monocrotophos (MCP) was stronger than Chlorpyrifos (CPF).

Table 1: Effect of Monocrotophos (MCP) and Chlorpyrifos (CPF) on MI of *Allium cepa* L.

Treatment	Concentration (%)	Total no of observed cells	Total no of dividing cells	Mitotic Index (MI %)	Prophase %	Metaphase%	Anaphase %	Telophase %
Control	-	2890	480	18.60 ± 0.21	14.84	13.84	1.41	0.97
CPF		2472	385	15.57 ± 0.66	11.36	2.32	1.22	0.67
MCP	0.12	2995	440	14.69 ± 0.16	9.76	2.80	1.40	0.73
CPF		2515	332	13.20 ± 0.05	9.20	2.06	1.26	0.68
MCP	0.25	2827	350	12.38 ± 0.11	8.82	1.86	1.06	0.64
CPF		2187	245	11.20 ± 0.02	8.60	1.10	0.94	0.56
MCP	0.50	3010	286	09.50 ± 0.21	7.30	1.03	0.86	0.35
CPF		2336	204	08.73 ± 0.15	6.70	0.94	0.79	0.30
MCP	0.75	2925	220	07.52 ± 0.05	5.49	0.88	0.74	0.38
CPF		2144	128	05.97 ± 1.02	3.88	0.97	0.74	0.38
MCP	1.00	3298	160	04.85 ± 0.91	2.67	0.94	0.82	0.42

Table. 2: Effect of Monocrotophos (MCP) and Chlorpyrifos (CPF) on mitotic chromosome of *Allium cepa* L.

Treatment	Concentration (%)	No. of dividing cells	% age of abnormal cells	Fragement %	Stickiness %	Diagonal Metaphase/ Anaphase/ Telophase %	Bridge %	Laggards %	Micronuclei / Binucleate %
Control	-	480	0.40	0.20	-	-	-	0.20	-
CPF		385	8.26	2.33	2.33	1.55	1.03	0.77	-
MCP	0.12	440	9.28	2.72	2.95	1.13	1.36	1.13	-
CPF		332	12.60	3.61	4.21	0.90	1.80	1.80	0.30
MCP	0.25	350	14.40	4.00	5.14	0.85	2.28	1.71	0.57
CPF		245	18.12	4.89	5.71	1.22	2.85	2.44	0.81
MCP	0.50	286	21.18	5.24	7.69	1.74	3.14	2.09	1.04
CPF		204	26.16	6.86	8.82	1.47	3.92	3.43	1.47
MCP	0.75	220	30.73	8.18	10.90	2.72	4.54	3.18	1.36
CPF		128	35.80	9.37	11.71	2.34	6.25	4.68	1.56
MCP	1.00	160	42.00	10.00	14.37	3.12	6.87	5.62	1.87

Discussion

In recent years several cytological studies have been carried out to detect the mito-inhibitory / genotoxic / cytotoxic / chromotoxic effects of various pesticides on different plants (Amar and Farah, 1974; Inceer and Beyazoglu, 2000; Lerda, 1992; Nandi, 1985; Kumar *et al.*, 2011). Most of these chemicals have been reported to have detrimental effect on the natural ecosystem and their residues in plants may also affect human health (Fujii and Inoue, 1983).

Mitosis: Mitotic inhibition by organophosphate pesticides has been attributed to blocking of mitotic cycle during interphase which may result from a prolonged G₂ period or arrest cell division at G₁ phase. Mohandas and Grant (1972) reported similar results by the application of herbicides. Reduction of MI might have been achieved by the inhibition of DNA synthesis at S-phase (Sudhakar *et al.*, 2001). Decrease in the mitotic index as a result of treatment with a particular substance shows its capacity to arrest cell divisions together with its ability to kill the actively dividing cells (Tajo and Thoppil, 1998).

The mitotic poison may cause metabolic imbalances which may interfere

with the synthesis, state and structure of nucleic acid including physiological effects and structural changes in chromosomes during cell division which may lead to mitotic delay and mitotic inhibition (Soni *et al.*, 1982). Many other investigations were attributed to depression of mitotic activity due to inhibition of protein synthesis (Kim and Bendixen, 1987).

Increasing concentration of the organophosphate pesticides interferes with the normal sequence of cell cycle that reduces the number of cell to enter prophase and succeeding divisional stages. Such type of mitodepressive action of chemicals have been reported earlier (de Campos and Viccini, 2003). Cumminis *et al.*, (1996) reported that the proteins which determine the duration of transition from metaphase to onwards are concerned with the transformation of chemical energy into the mechanical work of mitosis.

The result showed an inverse correlation between the organophosphate pesticides solutions and Mitotic Index (MI) which is compatible with the hypothesis that inhibition of mitosis may be due to inhibition

of DNA synthesis/Protein synthesis/ prolonged G₂ period.

Chromosomal abnormalities

The present investigation reports that organophosphate pesticides caused cytological changes and induced a wide range of mitotic abnormalities (stickiness, bridges, laggards, micronuclei, fragments etc) in the root tip cells of *Allium cepa* L. Our results matched with earlier findings made by (Ajay and Sarbhoy, 1987, El-Khodary *et al.*, 1989 and Kumar *et al.*, 2011; Sinha, 2009). Chromosomal abnormalities are considered as reliable indicators of mutational changes and are used as reliable evidence for screening the mutational activity (Kihlman, 1963).

Stickiness comprises the most dominant type abnormalities observed in all concentrations of the organophosphate pesticides. Stickiness may be considered to be a physiological effect exerted by pesticides (Grant, 1978). Patil and Bhat (1992) suggested that stickiness is a type of physiological adhesion involving mainly the proteinous matrix of chromatin material. The fragments has been observed in all concentrations of the Organophosphate pesticides, it may be formed due to DNA breakage by endonuclease (Grant, 1978). Alkylating agents are known to cause chromosomal fragments by binding to DNA region rich in GC pairs causing instability (Lawley, 1966) and Kaeppler *et al.*, 2000 has reported that the fragments may be the result of change in the levelk of DNA methylation. Multipolar anaphase abnormalities are caused due to inhibition of spindle formation (Amer and Ali, 1983). Bridges and Laggards were reported in all the concentrations. Bridges are caused due to breakage of chromosomes (Tomkins and Grant, 1972) while laggards due to stickiness of chromosomal end (Kaur and Grover, 1985). Micronuclei and Binudeate have been observed in both organophosphate pesticides. Micronuclei might be due to the aggregation of chromatin materials into masses of various number and size, Omanakumari *et al.*, (2006) reported that the micronuclei appeared due to fragmentations of chromosomes by the cacogenic action of monosodium glutamate and Ali *et al.*, (2008) observed micronuclei in fish by the treatment of chlorpyrifos.

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