RESEARCH ARTICLE

TOXICITY INDUCED BY PROPHENOPHOS AND CHLOROPYRIPHOS IN *LATHYRUS SATIVUS* L.

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Abstract: Dry seeds (moisture content: 3.22%) of grass pea (*Lathyrus sativus* L.; family: Fabaceae) are treated with different doses of (0.05, 0.1 ,0.2 and .03 percent, 3h) of pesticides namely Prophenophos (common name:Carina50) and Chloropyriphos (Dursban), and attributes like seed germination frequency, seedling length, mitotic index, chromosomal aberrations and total protein and soluble sugar content have been analyzed. The objective of this work is to foresee the extent of biological damages caused by the chemicals, which may enable to administer appropriate doses that cause lesser environmental hazards. Results have been analyzed.

Keywords: Pesticides, Chromosomal aberration, Biological damage, Environmental hazards

INTRODUCTION

Pesticides are used all over the world to protect agricultural products from pests, thereby stepping up the output for satisfying up surging demand of population. However, residues of these chemicals are hazardous to the health of nontarget organisms like domestic animals, human beings etc. (Ali 2007). Most of the pesticides are chromosome damaging as well as mutagenic and carcinogenic (Kihlman, 1966). The pesticides are introduced into the nature through plants and moulds (Ahluwalia, 1985) affecting the ecosystem resulting into environmental hazards. Therefore, careful assessment of such chemicals is of utmost importance. Plant system can be used as test material as it is convenient, easy to use and cost effective.

There is meager information regarding cytogenetic side effects of Prophenophos (common name: Carina50) and Chloropyriphos (common name: Dursban) the widely applied pesticides in West Bengal plains on a large variety of plants to control various types of insects in the fields. The present investigation has been undertaken to assess the toxic effects of these two pesticides on *Lathyrus sativus* L. (family: Fabaceae, common name: grass pea, 2n= 14) with a view to ascertain sub lethal doses, which can be beneficial to control environmental hazards.

MATERIAL AND METHOD

Dry and filled seeds (moisture content: 3.322%) of *Lathyrus sativus* L. (family: Fabaceae) were treated with different doses (0.05, 0.1 ,0.2 and 0.3 for 3h; doses administered were based on doses applied in the field by the farmers) of two pesticides namely Profenophos (common name:Carina50, formula: o-(4 bromo-2 chlorophenyl)-o ethyl 3 hydroxy propyl phosphorothioate, functions: causing stomach toxicity) and Chloropyriphos (common name: Dursban, formula: $C_9H_{11}Cl_3NO_3PS$, functions: on the nervous system of insects by inhibiting acetylcholine esterase) at $25^oC \pm 1^oC$ temperature and 80% relative humidity. The treated seeds were thoroughly washed in running water and were recovered in Knops solution for overnight and dried on blotting paper. Untreated control seeds were soaked in deionized water for overnight. Treated and control seeds were given in petriplates lined with moist filter paper under controlled condition ($25^o \pm 1^oC$). Germination frequency (bursting of seed coat and emergence of radical) and seedling growth were estimated from randomly taken 15 germinating seeds (7 days after sowing). Biological damages like lethality and injury were assessed from germination frequency and seedling growth respectively (Konzak *et al*. 1965).

For cytological studies suitable sized root tips from control and treatments were excised and fixed in acetic acid: ethyl alcohol (1:3) for overnight at room temperature. The fixed roots were then stained in 2% aceto orcein: 1(N) HCl(9:1) mixture and kept for 1hr. The root tips were excised and squashed in 45% acetic acid on a grease free slide and observed under microscope for scoring chromosomal aberrations and determination of mitotic index. Mitotic index (M.I.) was calculated from the following formula:

$$\text{Mitotic Index} = \frac{\text{Total no. of dividing cells}}{\text{Total no. of cells}} \text{X100\%}$$

Quantitative analysis of protein and total soluble sugar were made from seedlings of control and treatments (three replicas in each case) using the method described by Lowry *et al.*(1951) and Sadasivam and Manickam (1992) respectively.

RESULT AND DISCUSSION

Data relating to germination frequency, seedling growth and biological damages are presented in Table1. As compared to control, germination frequency is found to enhance in higher doses of

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Carina50. Among the treatments germination frequency varies from 20.0% to 40.0% (control: 28%). In Dursban, germination frequency shows dose dependent decrease (range:12.0% to 38.0%). Seedling growth has been noted to be 36.7mm + 0.21 in control and it shows significant reduction in treatment of both pesticides (excepting 0.3% carina50). Reduction in germination frequency and seedling growth has been attributed to the nature and extent of chromosome aberrations occurring in the cell (Mukherjee and Basu 1979, Datta and Biswas 1983, Datta *et al*. 1986) and to structural changes (Gray and Read 1950) of the chromosomes. Retardation of growth may primarily be due to the destruction of auxin at meristematic region (Singh 1974). In the present investigation lethality is less; although injury is predominant in treatment.

In the present investigation a total of 7 mitotic aberration types (Table2) is noted in dividing cells and 3 in resting cells. The aberrations types are: clumping and sticky nature of chromosomes (Fig.2) and fragment(s)[Fig.3] at metaphase, bridge(Figs.4-6) with or without accompanying fragment(s),early and late separation(Fig.7) of chromosomes and multipolarity(Fig.8) in anaphase cells. Micronuclei(Fig.9), giant cells and nuclear margination(Fig.10) are also recorded in resting cells. Control cells also reveal occasional occurrence of clumped metaphase, fragments, bridge and giant cell (Table2).Mitotic index is noted to be 19.63% in control; whereas, it ranges between 19.21% and 38.58% in Carina50 and between 18.49% and 41.395 in Dursban treatment. Mitotic index has been found to enhance in treatments of both the pesticides. The cell which is unable to repair the DNA damage has two options at the end of Hayflic limit – the first one is apoptosis by cytolysis second one is anapoptosis (a state of immortality) condition gained by the cell probably causing cancer (Sreedevi and Bindu 2004). Increase of mitotic index in this study, may be the result of anapoptosis condition earned by the cell when exposed in extremely stressed environment. Stickiness of the chromosomes at metaphase is the most common abnormality found in all treatments. Frequency of sticky cells shows dose dependent increase in concentration in both the pesticides. Stickiness may result from breakage and exchange between chromatin fibres on the surface of adjoining chromosomes (Abdelsalam *et al* 1999a). It causes inability of normal movement of chromosomes at anaphase (Sreedevi and Bindu 2004) resulting in nonsynchronus movement of chromosomes (Badr *et al.* 1985). Sticky chromosomes indicate high toxic chemical effects that are usually not reversible and will probably lead to cell death (Ateeq *et al*. 2002, Yuzba Sioglu 2003). Formation of metaphase fragments suggests breakages. Such fragments may be distributed randomly to either pole or may produce micronuclei at telophase (Abdelsalam *et al*. 1993b). Micronucleus and multipolarity are only found in Carina50 treated samples at higher doses. Occurrence of micronuclei has been regarded as reliable parameter for clastogenicity or mutagenicity of an agent (Auerbach 1976). The higher the micronucleus frequency detected in exposed organisms, the higher the potentiality of the agent to cause mutation (Sparrow 1961). Multipolarity is strongly correlated with bridges at anaphase which are due to dysfunctional telomerase produced by abnormal telomerase shortening (Gisselsson *et al.* 2002, Stewenius *et al.* 2007). Nuclear marginationis only found in Dursban treated samples.

Total Protein content (Table3) is found to decrease in lower doses of Carina50 treatment but increase in the applied dose. It indicates that initially gene expression was inhibited but at highest dose the plant tried to cope up with the adverse condition by inducing more proteins (probably stress protein). On the contrary, Dursban treatment shows initial increase in protein content but at higher doses it may be that initially protein (probably stress protein) production was induced to eliminate the stress given to it and ultimately at higher doses protein production decreases due to the lethal action of the chemical on gene expression. In Carina50 treatment total soluble sugar content (Table3) has been found to decrease in lower doses and increases in 0.3% concentration; while, in Dursban it increases in lower doses but abruptly decreases in 0.3% concentration. Increase of soluble sugar may be due to the breakdown of lipid content or may be due to the induction of gluconeogenesis.

Table1. Germination frequency (%), seedling growth and biological damage in petriplates for control and different doses of treatment.

Treatments (%)	No. of seeds given	Germination frequency(%)	Seedling growth(mm)		Biological damage(%)	
			Mean±SE	CV(%)	Lethality	Injury
Control	100	28.0	36.7±0.21	2.18	-	-
Carina50 0.05	100	20.0	18.75***±0.076	6.58	28.57	49.42
0.1	100	26.0	24.0***±0.073	5.59	7.14	33.43
0.2	100	24.0	12.63***±0.09	7.26	14.29	66.02
0.3	100	42.0	34.29±0.06	3.89	-	6.43

Dursban 0.05	100	38.0	28.44*±0.022	5.23	-	22.89
0.1	100	32.0	12.2***±0.02	7.34	-	17.69
0.2	100	22.0	30.42*±0.037	5.01	21.43	48.93
0.3	100	12.0	19.0***±0.15	6.08	57.14	67.008

t value at 28 DF
*- significant at 0.05 probability level, ***- significant at 0.001 probability level

Table 2. Mitotic aberration frequencies(%) and mitotic index(%) in control and in different doses of treatment.

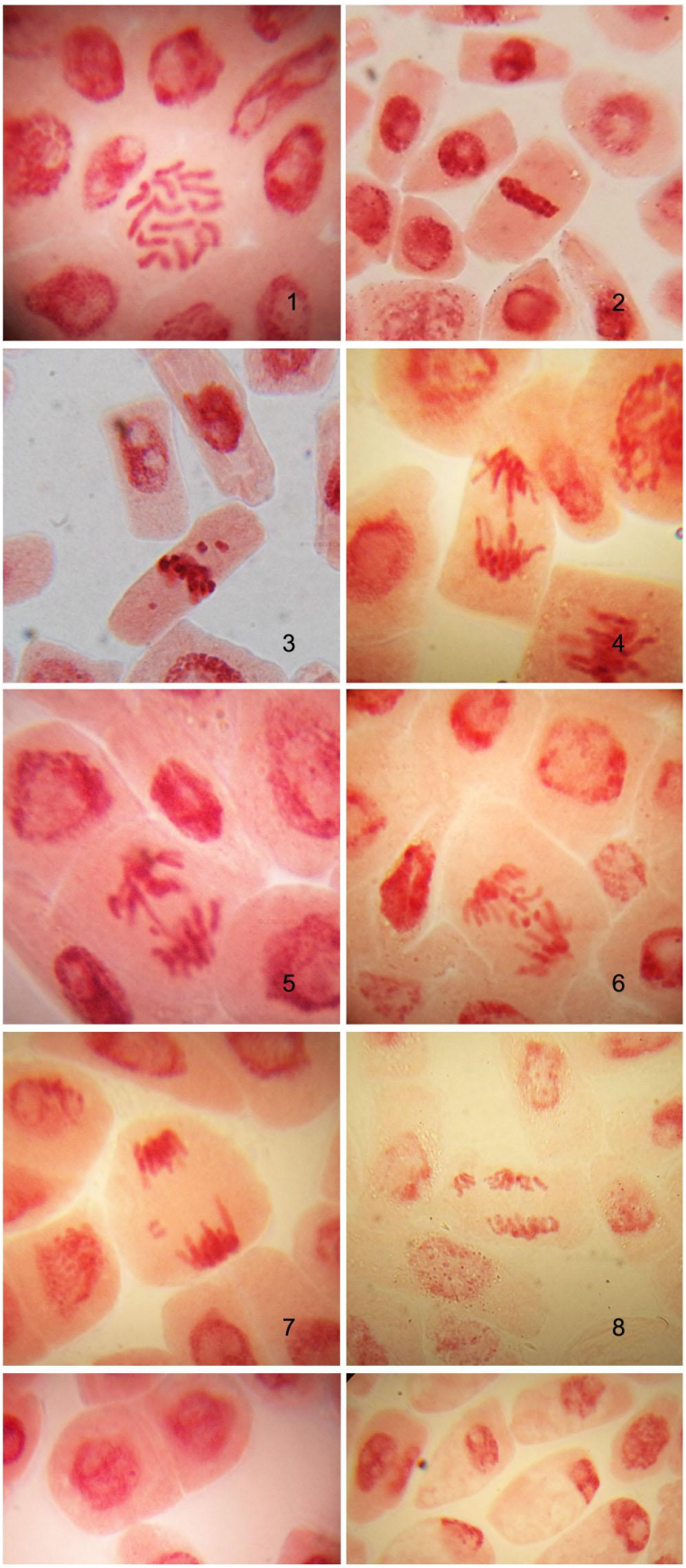
Treatments (%)	Total No. of cells scored	No. of dividing cells	Mitotic Index(%)	Frequency(%) of chromosomal aberrations									
				Dividing cells							Resting cells		
				CM	FM	ES	LS	BF	BWF	MP	MN	GC	NM
Control	1310	333	19.63	2.37	0.07	-	-	-	0.07	-	-	0.31	-
Carina50 0.05	1346	354	26.30	4.75	0.59	3.94	2.53	0.82	-	-	-	0.97	-
0.1	1275	245	19.21	8.16	1.65	2.43	4.78	1.65	1.65	0.78	-	1.02	-
0.2	1287	346	26.89	5.75	2.33	4.89	2.56	0.31	1.32	1.65	0.47	2.09	-
0.3	1174	453	38.58	16.5	3.07	3.49	3.92	3.32	1.96	-	-	2.81	-
Dursban 0.05	1058	438	41.39	10.9	0.47	2.08	2.46	1.13	0.95	-	-	1.61	-
0.1	1261	438	34.74	6.58	1.11	1.11	1.82	1.11	1.35	-	-	1.03	-
0.2	1175	345	29.37	8.42	1.45	1.70	2.64	1.45	1.45	-	-	0.94	0.43
0.3	1352	250	18.49	14.8	1.99	8.80	2.81	3.62	3.62	-	-	2.44	-

CM- Clumped and sticky metaphase, FM- Fragment(s) at metaphase, ES- Early separation, LS- Late separation, BF- Anaphase bridge with Fragment(s), BWF- Anaphase bridge without fragment, MP- Multipolarity, MN- Multinucleus, GC- Giant cell and NM- Nuclear margination,.

Table 3. Quantitative analysis of protein(%) and soluble sugar(%) from seedling of control and different doses of treatments.

Treatments	Protein(%)	Soluble Sugar(%)
Control	14.75	23.968
Carina50 0.05	9.71	12.32
0.1	12.88	20.72
0.2	12.32	19.88
0.3	17.08	40.32
Dursban 0.05	17.54	37.52
0.1	13.82	21.56
0.2	16.43	30.24
0.3	8.86	9.52

Explanations of the figures
Figs.1-10. 1-Normal metaphase, 2-Clumped and sticky metaphase, 3-Fragments at metaphase, 4-Anaphase bridge without fragment, 5- Anaphase bridge with fragment, 6-Broken bridge with fragment,7- Late separation of chromosomes, 8-Multipolarity, 9-Micromuclus and 10-Nuclear margination.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CONCLUSION

In this investigation both the insecticides show severe irreparable cytotoxic effect on *Lathyrus sativus* L., and both of them were found to be clastogenic (showing chromosomal aberration) and turbagenic (affecting the spindle) in nature. The toxicity increases mostly with an increase in concentration of the chemicals. Furthermore, micronucleus formation induced by Carina50 reflects the mutagenic nature of the chemical. The present investigation highlighted the necessity of proper monitoring of appropriate dose level in the pesticides to reduce environmental hazards.

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