
Research Article

Genotoxic effects of profenophos on *Pisum sativum*

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Abstract

Profenophos is a commonly used organophosphate pesticide in pulse crops; however, it is difficult to say whether it is safe from cytogenotoxic effects. Test plant materials (field pea seeds) were soaked in 250 ml of 0.2%, 0.4% and 0.6% profenophos 50% EC separately for 6 hours. The mechanism behind various types of chromosomal anomalies observed due to treatment with profenophos has been discussed in detail. The effects of the pesticide that appeared in M₁ generation diluted in M₂. The appearance of C-metaphase with univalent and bivalents, multipolarities, chromatin bridges suggest that these organophosphate pesticides like profenophos affect genetic recombination which may lead to loss of important factors, gain of undesirable characters. This study aims at finding the cytological and genotoxic effect of pesticide profenophos on germ cells of *Pisum sativum* var. *arvense* and suggests judicious means of application of pesticides and agrochemicals in appropriate condition to elude further damages in the future.

Introduction

Pisum sativum (field pea) is one of the oldest domesticated and cultivated legume crops in the world. It is grown mostly in the tropical and temperate regions. In 2017, a total of 8,141,031 hectares of field pea were harvested globally, with the top producers consisting of Canada, Russia, China, India, and the United States (Food and Agriculture Organization, 2019). Field pea is an important source of plant protein (protein level 23-33%) for human as well as animals.

Among various leguminous crops grown in India field pea (*Pisum sativum* L. var. *arvense*) holds an important position as a pulse crop. In

India, it is grown mostly in the *rabi* (winter) season. It belongs to tribe- Viciaeae, order- Fabales, family- Leguminosae (Fabaceae), subfamily- Papilionaceae, genus- *Pisum* and species- *sativum* with chromosome number 2n = 14 and has a plethora of utilities as food, feed and fodder.

Pesticides play an important role in modern intensive agriculture. They help to keep the pest population below the economic threshold level and thereafter increase crop yield (Ouyang et al., 2013). Although they are meant to be target-specific, their residual toxicity affects other non-target plant and animal species at cellular and molecular levels. Some of these

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pesticides are chemical mutagens and thus seek considerable attention to avert the damages caused by them on physiological and cellular systems of plants and other organisms (Sharma et al., 2016; Shahzad et al., 2018). Chromosomal abnormalities and pollen sterility on treating with herbicides monochloroacetic acid and trichloroacetic acid were reported by (Bonciu, 2012; Maity, 2014; Pulate & Tarar, 2014; Aksoy et al., 2015) reported that carbamates have the ability to induce chromosomal aberrations. Choudhary and Sajid (1986) noted the changes in chiasma frequency up to M_2 generation in two varieties of pea plant when treated with bavistin, a fungicide. Jain and Sarbhoy (1988) demonstrated the cytogenetic effects of benzene hexachloride (BHC) lindane, aldrin, heptachlor and, endrin on several leguminous plants like *Lens culinaris*, *Lens esculenta*. So, to understand the genotoxic effects of the pesticides it is essential to perform cytogenetic screening of standing crops. The present study delineates the cytological effects of profenophos, an organophosphate pesticide on the behaviour of meiotic chromosomes in both M_1 and M_2 generation plants.

Materials and methods

Experimental site

The field experiment for the investigation was carried out during *rabi* season of 2018-19 and 2019-20 at an experimental field in Chandamari, Nadia, West Bengal, India. The area lies under the New Alluvial zone of West Bengal which is characterized by high rainfall (1300-1600 mm annually), high relative humidity and moderate temperature. The topography of the land where experiment has been conducted was low to medium in situation. The soil was clay loam in nature with higher proportion of silt, having good water holding capacity with relatively neutral pH (6.0-6.5). Zn status is near the critical level.

Plant materials

Certified seeds of *Pisum sativum* var *arvense* variety HUDP15 (Malviya Matar 15) released by BHU Varanasi, U.P., India were used as plant material for the experiment.

Treatment details

50 seeds each of the experimental plant material were soaked in 250 ml of different concentrations (0.2%, 0.4%, and 0.6%) of profenophos 50% EC for 6 hours. Seeds soaked in distilled water were used as control.

Experimental design

The seeds were sown separately in field plots following the Randomised Block Design (RBD) having 3 replications, maintaining a row to row spacing of 25 cm and a plant to plant spacing of 15 cm.

Cultural practices

Seeds were sown by the middle of October and harvested in February. Irrigation and other operations were performed without the application of manures. First irrigation was applied during pre-flowering stage and second at pod formation. Two weedings are done; first one at 2-3 leaves stage or 3-4 weeks after sowing and the next one is done before flowering.

Data collection and analysis

Plants obtained in the M_1 generation were analysed for the rate of induced variability. Seeds from these plants were collected and further sown to raise the M_2 generation. The quantitative characters assessed in M_1 were analysed for M_2 as well.

For meiotic study flower buds of suitable size of both controlled and treated plants were fixed in Carnoy's fixative (Absolute alcohol: Chloroform: Glacial acetic acid-6:3:1) and smeared in 2% acetocarmine. Photomicrographs were taken from suitable plates.

Results and discussion

Field study

Phenotypic effects on plants of M_1 and M_2 generations were assessed by their mean and corresponding coefficient of variability (CV) values based on several yield attributing parameters as mentioned in Table 1 and Table 2. The mean values decreased considerably with the increase in the concentration of pesticide solution showing a linear dose response relationship. In M_1 generation the mean values depicting plant height (54.24 cm to 57.28 cm),

number of branches per plant (26.16 to 29.12), number of leaves per plant (125.26 to 145.52), number of flowers per plant (12.16 to 12.06), number of pods per plant (11.42 to 11.62), pod length (5.56 cm to 6.20 cm), number of seeds per pod (5.15 to 5.72), yield per plant (40.62 g to 48.44g) showed the maximum for 0.2% of profenophos with respect to the control.

Meanwhile, the CV values oscillated irregularly with the increase in the concentration of pesticide solutions. Similarly in M_2 generation, maximum mean values were noted for 0.2% of pesticide solution (profenophos) and the values gradually decreased with the increase in its concentration for all the parameters examined.

Table 1. Phenotypic effect of profenophos on M_1 parameters in *Pisum sativum* var. *arvense*

Doses of profenophos	Plant height (in cm)		No. of branches per plant		Number of leaves per plant		Number of flowers per plant	
	Mean± SE	CV %	Mean± SE	CV %	Mean± SE	CV %	Mean± SE	CV %
Control	57.28 ± 1.267	7.402	29.12±1.166	11.208	145.52±4.338	12.081	12.06± 1.388	18.024
0.2%	54.24±1.560	8.862	26.16± 1.128	10.166	125.26± 1.282	8.066	12.16± 1.261	17.662
0.4%	48.12±1.166	7.681	17.40±1.116	09.062	115.22±1.202	9.382	09.88±0.766	14.324
0.6%	34.24±1.312	8.277	12.48±0.819	11.188	98.06±2.066	12.124	09.02±0.458	11.064
Doses of profenophos	No. of pods per plant		Pod length (in cm)		No. of seeds per pod		Yield per plant (in g)	
	Mean± SE	CV %	Mean± SE	CV %	Mean± SE	CV %	Mean± SE	CV %
Control	11.62± 0.629	18.06	6.20±0.215	10.252	5.72±0.216	11.262	48.44±0.982	14.266
0.2%	11.42±0.812	06.03	5.56±0.312	18.151	5.15±0.355	10.166	40.62±0.824	06.262
0.4%	10.56±0.414	08.67	4.48±0.218	15.316	4.12±0.288	14.103	29.16±0.767	07.233
0.6%	08.56±0.652	10.22	3.52±0.251	14.376	3.44±0.216	14.316	26.24±0.408	12.262

Table 2. Phenotypic effect of profenophos on M_2 parameters in *Pisum sativum* var. *arvense*

Doses of profenophos	Plant height (in cm)		No. of branches per plant		Number of leaves per plant		Number of flowers per plant	
	Mean± SE	CV %	Mean± SE	CV %	Mean± SE	CV %	Mean± SE	CV %
Control	59.20±1.281	7.488	29.66±1.520	12.166	144.28± 4.414	12.066	12.66±1.414	16.221
0.2%	56.32± 1.626	8.814	28.42± 1.208	10.109	128.02± 2.180	10.034	12.18±1.664	14.816
0.4%	50.25±1.116	7.882	22.54±1.212	09.080	116.08± 2.062	09.484	10.16±1.068	13.266
0.6%	38.24±1.566	8.216	18.62± 0.679	09.316	92.04±1.366	11.216	09.45±1.388	10.082
Doses of profenophos	No. of pods per plant		Pod length (in cm)		No. of seeds per pod		Yield per plant (in g)	
	Mean± SE	CV %	Mean± SE	CV %	Mean± SE	CV %	Mean± SE	CV %
Control	11.68±0.459	16.072	6.38±0.216	10.202	5.68±0.217	11.266	52.06±0.816	14.026
0.2%	11.60±0.258	8.086	6.06±0.318	14.266	5.22±0.256	10.382	50.27±0.562	12.034
0.4%	10.54±0.168	7.686	5.50±0.312	14.364	4.92±0.343	12.266	38.86±0.638	8.034
0.6%	09.52±0.456	8.443	4.46±0.288	12.308	3.97±0.282	12.106	30.29±0.546	9.206

Meiotic study

Chromosomal abnormalities observed in the cells of flower buds of M_1 due to application of organophosphorus pesticide, profenophos were clumping, grouping, stickiness, breakage, anaphasic bridge or laggards, micronuclei, C-metaphase with bivalents, binucleate cells, multipolarity, and cytotoxic cells (Figure 1).

Such chromosomal anomalies similar to those identified in the present study, resulting from treatment with pesticides, were noted by other authors as well (Haiba et al., 2011; Pulate & Tarar 2014; Selvaraju et al., 2015). Out of these, clumping, grouping and stickiness were most common.

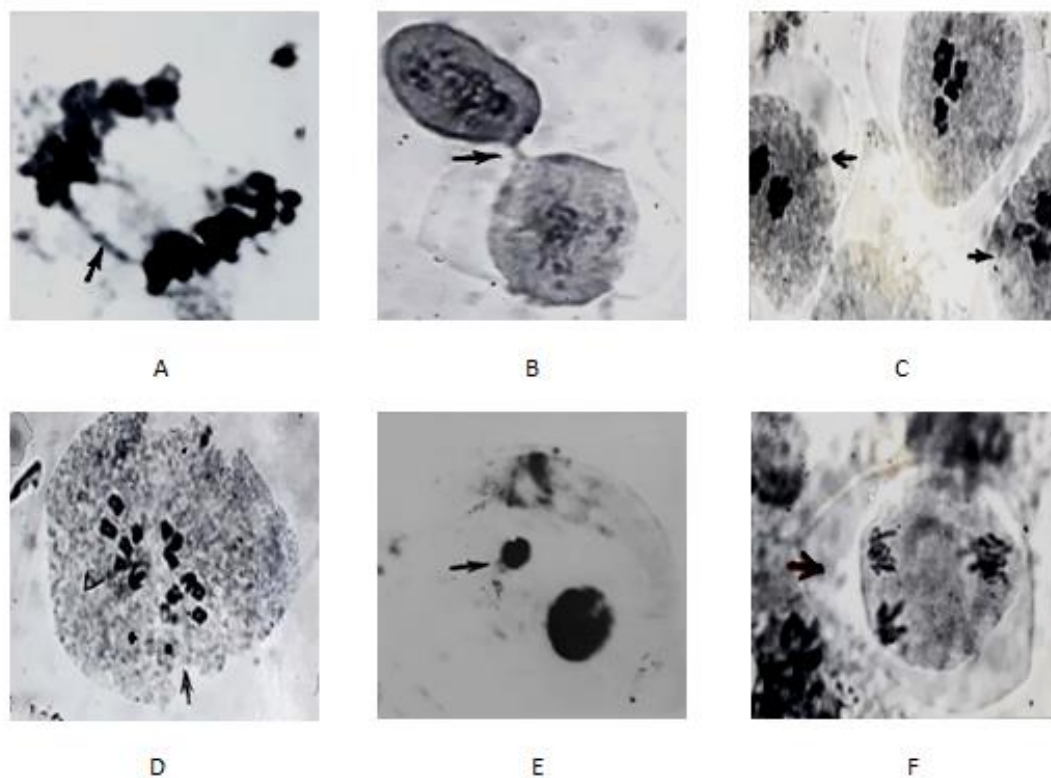


Figure 1. Meiotic abnormalities induced by pesticide profenophos in *Pisum sativum* var. *arvense*.
 A. Anaphasic bridge, B. Cytomixis, C. Clumping, D. C-metaphase, E. Micronuclei, F. Multipolarity

Clumping was noted maximum (10.56%) at 0.6% concentration and minimum (4.08 %) at 0.2% with respect to control (0.18%). Similar anomalies were noted for cells from the M_2 generation (plants obtained from the seeds of M_1 generation). Again, clumping was found to be the highest (6.26 %) at 0.6% as compared to

control (0.22%). Cytomictic cells were also noted in both M_1 and M_2 generations showing the maximum values at higher concentration of treatments. The percentages of different chromosomal anomalies (mean $\pm$ SE) for each treatment in M_1 and M_2 generations are presented in Table 3 and Table 4.

Table 3. Meiotic chromosomal abnormalities (in percentage) in M_1 induced by the effect of profenophos in *Pisum sativum* var. *arvense*

Doses	Clumping or grouping or Stickiness	Micro-nuclei	Break age	Anaphasic bridge and laggards	C- Meta-phase	Binu- cleate cell	Multi- polarity	Cyto- mixis	Total anomalies
Control	0.18	ND	ND	ND	0.14	0.16	ND	0.28	0.76 ± 0.112
Profenophos									
0.2%	4.08	ND	ND	ND	0.12	0.44	0.28	0.42	5.34 ± 0.086
0.4%	6.14	0.46	0.58	0.36	0.14	0.55	ND	ND	8.23 ± 0.264
0.6%	10.56	0.24	1.24	0.85	ND	0.34	ND	0.68	13.91 ± 0.118

ND: Not detected

Table 4. Meiotic chromosomal abnormalities (in percentage) in M_2 induced by the effect of profenophos in *Pisum sativum* var. *arvense*

Doses	Clumping or grouping or stickiness	Micro-nuclei	Breakage	Anaphasic bridge and laggards	C-Metaphase	Binucleate cell	Multi-polarity	Cytomixis	Total anomalies
Control	0.22	ND	ND	ND	0.28	ND	ND	0.16	0.66 $\pm$ 0.082
Profenophos									
0.2%	3.76	0.28	0.24	ND	ND	ND	ND	0.28	4.56 $\pm$ 0.115
0.4%	4.69	0.32	0.82	0.20	0.29	0.27	ND	0.40	6.99 $\pm$ 0.216
0.6%	6.26	ND	2.06	0.87	ND	0.63	ND	0.75	10.57 $\pm$ 0.324

ND: Not Detected

Discussion

From Table 1 and Table 2 representing the yield attributing quantitative characters, it can be said that the effects of pesticide were slightly diluted in the M_2 generation. Similar results were reported by Haiba et al. (2011) on *Vicia faba* due to spraying with fungicide Dithane M 45. From present study, it can be inferred that prolonged application of these organophosphate pesticides in high concentration may induce genetic damage in pure germ line.

Table 3 and Table 4 delineate the results obtained from the meiotic study depicting the changes that occurred to the plants at the cellular level when treated with different concentrations of profenophos. The most common anomalies observed were clumping, grouping or stickiness. The other anomalies like micronuclei, chromosome breakage, anaphasic bridge or laggards, C- metaphase with bivalents, binucleate cells, cytotoxic cells, etc. were also noted. According to Darlington (1953), stickiness arises from the diplomerisation of DNA on the surface of chromosomes. Stickiness was also observed in *Allium cepa* due to pesticide application by Kuchy et al. (2015). Partial dissolution of the chromosomes prevented proper functioning of the spindle apparatus responsible for the regular separation of the chromosomes in anaphase resulting in coalescence and clumping of the chromatin material. Many of the anthropogenic stresses including agrochemicals and fungicides were reported to cause spindle aberration during the first and second meiotic division in some cases gave rise

to unreduced gametes and the potential for polyploidisation (Mason & Pires, 2015; Fuchs et al., 2018), though it has been infrequently observed in our investigation. Breakage might be the result of the upset of the nucleic acid metabolism manifested by disturbed protein reduplication causing chromosomes to break at different loci (Darlington, 1953). Breakage was due to misrepair of DNA responsible for linear continuity of chromosomes, however no definite cause has yet been figured out. The occurrence of micronuclei is a reliable parameter for clastogenicity or mutagenicity. Pollen mother cells showing bridges were also encountered at anaphase-I. However, their frequencies were less compared to the other types of anomalies.

The cells showing cytomixis were generally observed at anaphase-I, occasionally at metaphase I. This leads to the loss or gain of chromatin in some cells. This chromatin loss which was not lethal might have resulted in the formation of nuclei with varying chromosomal constitution as was evident by the presence of multipolarity. Multipolarity is determined by position and number of poles which again depends on the position of the aggregation of RNA and polysaccharides which remain suspended in the form of sol or gel (Kumar et al., 1978). The presence of binucleate cells reflects the occurrence of either cytomixis or synodiploidy. Two pollen mother cells fuse followed by mixing of cytoplasm leading to the formation of a binucleate pollen mother cell. These suggest that certain structural changes might occur to chromosomes under the influence of pesticides.

Such changes may become permanently established within the species population either in the homozygote or heterozygote forms. However, it can be observed that the cytological abnormalities tend to dilute in M_2 generation compared to M_1 (Figure 2). This indicates the

recovery phenomenon due to *in situ* gene regulatory mechanisms. Therefore, it is essential to conduct routine surveys in the fields where pesticides are applied to figure out whether their applications are causing lethal genetic mutations in plant communities.

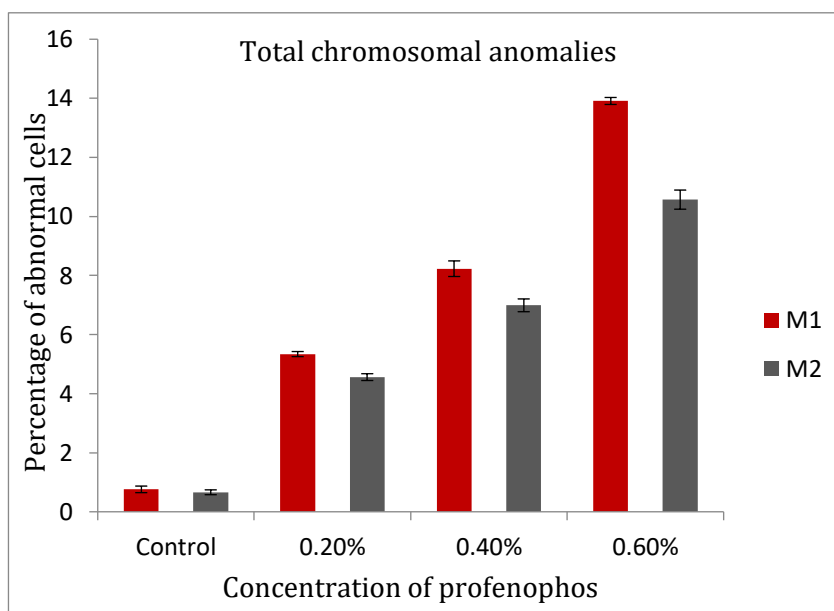


Figure 2. Comparative analysis of chromosomal abnormalities observed in meiotic cells by the effect of profenophos in M_1 and M_2 generation

Conclusion

Based on the present experiment and the foregoing discussion it stands out that to minimize the hazardous effects of pesticides and other agrochemicals, more research efforts should be taken to develop eco-friendly crop protection chemicals of both synthetic and natural origin. Endeavours are needed to develop botanical pesticides and sustainable bio-control measures. Special attention has to be given to the development of indigenous technologies for producing environmentally benign chemicals to ensure food security. Legislations that encourage the use of botanicals and eco-friendly farming practices are to be enforced to protect the environment and public health.

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Author's declaration and contribution

Authors declare no conflict of interest. Both the authors contributed equally to prepare this manuscript.

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