

Pest management in pulses and genotoxic effect of some pesticides in *Pisum sativum var. arvense*

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Abstract

Pulses are one of the most important crops grown in India. Every year pulses are attacked by various insect and non-insect pests either in field or at storage. Chemical pesticides are very effective in controlling pests due to their quick mode of action and residual toxicity, but these chemical agents have serious clastogenic and cytotoxic effects on the crop as well. A descriptive field survey was conducted in two pulse growing districts of West Bengal, India to figure out the commonly used pesticides for pulse crop cultivation in these regions. From them three such pesticides Profenophos, Spinosad, Dimethoate were selected and germinated seeds of field pea (*Pisum sativum var. arvense*) were treated with them. Mitotic preparation were made following the standard aceto-orcein technique and assessed. The study revealed that the use of these chemical pesticides can reduce Mitotic Index (MI) and induce genotoxic effects showing chromosomal abnormalities.

Keywords: Pulses, chemical pesticides, mitotic index, chromosomal aberration, genotoxic effect

1. Introduction

Pulses cater the protein requirement of the majority of Indian population and play a significant role in ensuring the nutritional security of the country. Because of their ability to fix atmospheric nitrogen in soil they used as green manure. Pulses also play a prominent role in agricultural system as cover crops in short season cropping windows, breakfast grains and dietary element. Pulses as a complement to cereals, make one of the best solution to protein-calorie malnutrition [1]. A variety of pulse crops including field pea, chick pea, mung bean, urad bean, lentil, french bean, horse gram, moth bean, grass pea are grown in different parts of the country. By 2030 and 2050 the pulse requirements of the country are estimated at 22.5-32.0 and 39.0 million tonnes, respectively. For this, an annual growth rate of over 2% is to be achieved [2]. Insect pest and diseases has always been a major threat causing significant losses in the yield. With the changes in climate and cropping systems, the pests have become more invasive and new pests having resistance to common pesticides have evolved. Some pests attack the crops more in the field during development while others attack in storage. Limited number of studies conducted, indicated yield loss in the field ranging from 15.6 to 67.8% for a pest complex and from 33.5 to 62.5% for specific pest species; meanwhile, losses in storage average 26.3% [3]. In modern agricultural system different types of pesticides are widely used to control the insect and non-insect pests that can remarkably reduce the amount of harvestable produce reducing the issue of crop losses to a great extent. Pesticide residues are known to persist in soil [4], water and in fruits and vegetables [5] and pose a serious threat for plant and human health. Genotoxicity and mutagenicity of pesticides on non-target organisms and their influence on ecosystem are of global concern [6]. Our investigations have been focused only to the usage of some chemical pesticides on pulses in West Bengal and their genotoxic effects in *Pisum sativum var. arvense* as pulse crop.

2. Materials and Methods

A descriptive survey was performed in Nadia and Murshidabad districts of West Bengal, India to find out the most devastating pests of pulse crops and the pesticides used to control them. Three pesticides Profenophos, Dimethoate and Spinosad were selected for study. The clastogenic and cytotoxic effects of the pesticides were analysed on the root meristem of *Pisum sativum var. arvense*. The growing root meristems of *Pisum sativum var. arvense* were immersed in Profenophos, Spinosad and Dimethoate solutions of 0.2%, 0.3% and 0.4% each. For each treatment, ten germinated seeds were transferred onto filter paper soaked in the pesticide solutions in petridishes and kept for 6hrs at room temperature. Distilled water was used as a negative control. After the mentioned period of exposure, three root tips from three seedlings per treatment were collected at random. Excised root tips were fixed in 1:3 acetic alcohol after that, mitotic preparation were made following the standard aceto-orcein squash technique and assessed. All the results were expressed as the mean of three replicates per sample \pm Standard Error (SE).

3. Results and Discussion

In West Bengal pest populations are dynamic, responding to climatic changes and misuse of protection practices. This led to the evolving new and often more damaging variants. Yield losses due to pests have been estimated at 10-35% annually. Pesticide consumption increased many folds with the introduction of high yielding varieties. The country manufactures all the conventional formulations including wettable powders (WP), Water Suspension Concentrate (WSC), Emulsifiable Concentrate (EC) or emulsion concentrate, soluble liquid (SL), dusts, aerosols. The pesticides are to be used only at recommended doses against a specific crop pest. Out of the survey conducted it was found that aphids, thrips, pod borers, pod bugs are the most devastating pests of pulses in West Bengal.

The commonly used pesticides were taken into account (Table1).

Table 1: Some commonly used pesticides in pulse crop cultivation in West Bengal and their target pests.

Insecticide	Group	Application method	Formulation, dose	Pests controlled
Difenthiuron	Carbodiimide	Foliar spray	500EC 12.5g a.i./ha	Whiteflies
Dimethoate	Organophosphate	Seed treatment	30EC 1.5g/kg	Thrips, white flies, pod bugs, aphids, pod borers
		Foliar spray	3EC 50,100, 150g a.i./ha	
Monocrotophos	Organophosphate	Foliar spray	36SL, 150,225g a.i./ha	Sap sucking pests pod bugs
Profenophos	Organophosphate	Foliar spray	40EC, 400 g a.i./ha	Jassids, thrips, whiteflies
Spinosad	Naturalyte	Foliar Spray	45SSC, 150.7 g a.i./ha	Aphids, thrips, whiteflies
			48SC, 72,75.15 g a.i./ha	Pod borers, pod bugs
Thiamethoxam	Neonicotinoid	Seed treatment	70WS, 2-6g/kg	Aphids, jassids
		Seed treatment	35FS,3.5g/kg	Bean stem fly
		Foliar spray	25WG, 50-150 g a.i./ha	Aphids, thrips, whiteflies

The most probable reason behind genetic damages due to genotoxic agents in somatic as well as germinal cells is cell division abnormalities [7]. Mitotic Index (MI) end point is a reliable indicator of the presence of cytotoxic pollutants in the environment. On each of the three slides for each concentration, a total of 1,000 cells, classified into dividing cells (prophase, metaphase, anaphase or telophase) were scored. The MI was expressed as the number of dividing cells per 1,000 cells scored.

The mitotic index of each treatment was compared with that of the negative control group. The three pesticide solutions caused diverse chromosomal aberrations in mitotic cells of *Pisum sativum var.arvense*. The frequencies of such aberrations were quite high than those of control showing a linear dose response relationship. The data on the impact of pesticide solutions on the Mitotic Index (MI) in root meristem of *Pisum sativum var. arvense* are presented in Table: 2.

Table 2: Number of dividing cells (NDC) and Mitotic Indices (MI) of *Pisum sativum var. arvense* root meristematic cells exposed to Profenophos, Spinosad, Dimethoate after different concentrations of treatment.

Sample	Concentration of Treatment (%)	(NDC± SE)	Prophase	Metaphase	Anaphase	Telophase	(MI±SE)	MI% to Control
Control	-	174 ± 9.8	60.2	58.7	34.2	21.3	17.4 ± 0.44	100
Profenophos	0.2	160 ± 8.2	58.4	54.6	31.2	15.8	16.0 ± 0.42	91.9
	0.3	102 ± 6.8	38.8	30.8	20.6	11.8	10.2 ± 0.43	58.6
	0.4	90 ± 7.2	30.2	30.6	18.2	11.0	9.0 ± 1.6	51.7
Spinosad	0.2	154 ± 6.6	50.2	48.5	30.6	25.1	15.4 ± 0.26	88.5
	0.3	98 ± 5.8	35.4	30.4	18.7	13.5	9.8 ± 0.14	56.3
	0.4	82 ± 4.9	26.8	24.2	16.4	14.6	8.2 ± 0.22	47.1
Dimethoate	0.2	148 ± 7.4	50.8	46.3	26.4	24.5	14.8 ± 0.24	85.0
	0.3	88 ± 6.4	30.8	32.2	14.6	10.4	8.8 ± 0.12	50.5
	0.4	76 ± 4.2	26.1	22.4	12.8	14.7	7.6 ± 0.11	43.6

A notable decrease of Mitotic Index (MI) in all concentrations was detected, that is significantly different from control: reduction of 8.1- 48.3 % for Profenophos, 11.2- 52.9% for Spinosad and 15- 56.4% for Dimethoate. The MI depicted a sharp decline when the root tips were treated with 0.4% pesticide solutions. Prominently, MI endpoint was dose-dependent (Fig 1), but here, the decrease effect normally correlate with the pesticide concentration. Significant changes in all phases of mitotic cell division

indicated remarkable irregularities in root cell cycles. The results exhibited accretion of prophase stage, about an inhibition of mitosis or extension of cell cycles. This may be due to the blockage of cell division at the end of the prophase stage. In this regard the pesticides may be considered as pre- metaphase inhibitors. Mito-depressive action on the cell division may be the reason of MI reduction in *Pisum Sativum var. arvense* roots.

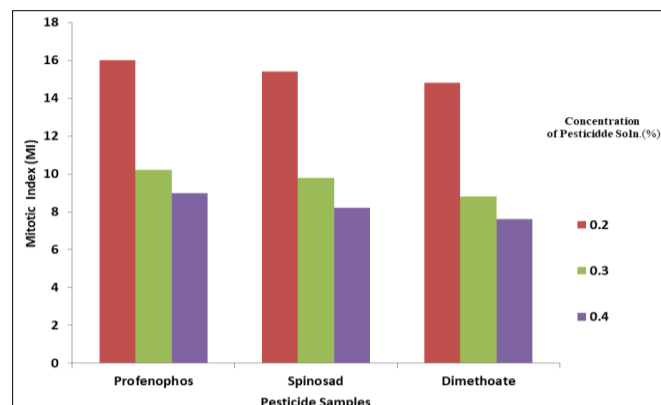


Fig 1: Comparative account of MI due to effect of pesticides in *Pisum sativum var. arvense*

Various types of chromosomal aberrations were induced by the three pesticide solutions. The mostly observed ones were stickiness, c-metaphase, and laggard chromosomes (physiological abnormalities) and fragment, bridges and

multipolarity (clastogenic ones) as shown in Table 3: Sporadic chromosomal loops, abnormally uncoiling in telophase, ring chromosomes and polyploidic cells were also observed [8].

Table 3: Frequency of abnormal cells and chromosomal aberration types of *Pisum sativum var. arvense*

Sample	Concentration of Treatment (%)	Abnormal Cell (%)	Stickiness/Clumping (%)	C-Metaphase/Diplo-chromosome (%)	Multipolarity (%)	Binucleate Cell (%)	Micronuclei (%)	Anaphasic/Telophasic Bridge (%)	Fragment/Laggard (%)
Control	—	1.8	0	0.2	0	0	0	1.8	0
Profenophos	0.2	5.2	1.6	0.6	0	0.8	0	1.4	0.8
	0.3	12.6	2.1	2.0	1.8	1.6	0.4	2.8	4.9
	0.4	15.6	6.6	0.4	2.4	0	0.6	1.2	4.4
Spinosad	0.2	6.8	0.8	0.6	0	2.4	0	1.8	1.2
	0.3	12.5	4.1	0.5	1.2	0	0	4.2	2.5
	0.4	14.8	4.0	2.1	0	1.8	1.0	2.8	3.1
Dimethoate	0.2	6.2	1.0	1.2	0	0.6	0	1.6	1.8
	0.3	7.4	2.6	0.6	1.6	0	0	1.4	1.2
	0.4	16.2	1.8	0	2.8	0.8	1.6	2.6	6.6

For each of the three slides for a particular dose, 100 cells, that is, 300 cells per pesticide treatment were examined for chromosomal aberrations (Fig: 2). The following types of aberrations were observed and scored (Table:3, Fig:2)

- Chromosome fragments: Pieces of chromosome lacking centromere; broken from total chromosome as a result of pesticide treatment (Fig.2D).
- Anaphase or Telophase Bridge: Dicentric chromosomes that form a bridge between both poles at anaphase or telophase. Generally it indicates paracentric inversions or other possibilities that include breakage and fusion of chromosomes and sister chromatid reunion (Fig.2G).
- Laggard: Entire chromosomes that fail to migrate to either pole at anaphase because of damage to the centromere and/or kinetochore (Fig.2F).
- C-Metaphase: Mitotic cells that lack spindle fibres so that the chromosomes remain scattered throughout the cell. In general it is seen in cells treated with the spindle poisons, colchicines or colcemid (Fig.2B).
- Multipolar anaphases and telophases (Multipolar): Anaphase and telophase cells with three or more spindle poles instead of the normal two. It is caused by damage to the centrosome.
- Sticky chromosomes: In this case the chromosomes fail to condense completely so that at metaphase, the chromosomes are still long like prophase chromosomes and remain entangled with each other.
- Micronuclei: Micronuclei are resulted from acentric fragments (Clastogenic effect) or the entire chromosome that through incomplete migration have been excluded from the main core (aneugenic effect). Thus micronuclei represent as loss in chromatin as a result of damage to either chromosome structure (fragmentation) or mitotic apparatus (Fig.2C). There may also be formation of bilobed nuclei (Fig.2E) thereby indicating an early change in cellular metabolism [9].

In extreme cases, chromatin masses are almost indistinguishable as chromosome are seen as clumps (clumping-Fig.2A), disruption of spindle fibres also produced diplocentric chromosomes (Fig: 2H) besides C-metaphase, failure of cytokinesis sometimes developed

binucleate cells. Similar observations were reported by Parry *et al* [10].

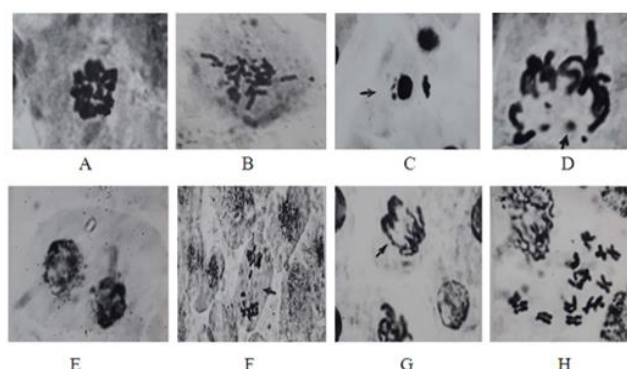


Fig 2: Mitotic Chromosomal abnormalities found due to genotoxic effect of pesticides in *Pisum sativum var. arvense*- A: Clumping, B: C- Metaphase, C: Cell with micronuclei, D: Chromosomal fragment, E: Binucleate Cell, F: Anaphasic laggard, G: Anaphasic bridge, H: Cell with diplochromosomes.

This study revealed that the 3 pesticides are quite similar to alkylating agents and radiomimetic agents in their cytological effects in the test material. Owing to the wide application of these pesticides in agricultural practices further investigations on a more intensive and extensive basis are required, as the final recipients of these chemical agents are none but the crop plants and their progeny.

4. Conclusions

As compared to the other control measures the chemical pesticides are more extensively used in pulse crop cultivation because of their quick knockdown effect and residual toxicity.

Biological agents, although relatively environment friendly in contrast with conventional pesticides, are not always available to the farmers. The results of this study revealed that the three pesticides, commonly used in Indian agriculture, can potentially induce cyto- and genotoxic effect on crops and ultimately damage plant, animal and human health. Thus the result may be taken as a warning or indicator that the chemical pesticides, although a short term remedy, may be a potent risk to the crop health in general. The data obtained from this experiment might be considered

preliminary and should be followed by further *ex-situ* investigations.

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