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CHROMOTOXIC AND MITODEPRESSIVE ACTIVITIES INDUCED BY TWO PESTICIDES; ENDOSULFAN AND CHLORPYRIFOS IN *VIGNA UNGUICULATA* L.

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The present study deals with the effect of pesticides *viz.* Endosulfan and Chlorpyrifos on somatic cells of *Vigna unguiculata* L. The root tips were treated with 50, 100, 150 and 200ppm concentration of both the pesticides for 5h. The different types of chromosomal aberrations observed which include, C- metaphase, precocious movement, unoriention, disturbed metaphase, lagging chromsome, ring formation bridges and others. The mitotic indices were determined and chromosomal abnormalities were investigated in different test groups of both the pesticides. It was found that Endosulfan was comparatively more chromotoxic and mitodepressive than Chlorpyrifos.

Keywords : Chlorpyrifos; Chromosomal abnormalities; Endosulfan; Mitotic index; *Vigna unguiculata.*

Introduction

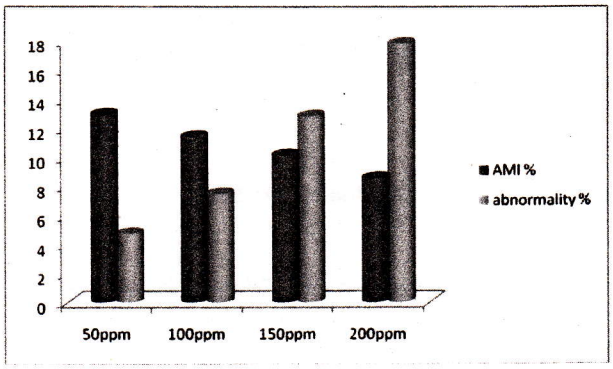
Vigna unguiculata (cowpea, 2n=22) is one of the major pulse crop of the Kharif season (July-September) in India. It is infested by all the major pests of pulses, namely pod borers, aphids and hairy caterpillar, which cause reduction in yield[1]. Numerous pesticides are extensively used in mordern agriculture practices for disease control and many cytological investigations have been carried out to detect the harmful effect of various pesticides on different plants[2-4]. The use of pesticides in current agricultural practices is steadily increasing. The efficiency of these pesticides in the better exploitation of plant species of economic importance is well known. However the potentialities of these pesticides as mutagenic and /or carcinogenic agents to the non-target organisms, as has been demostrated by several workers[5-7]. Although the use of pesticides has become esssential for disease control but their ingredients have induced acute toxic effects[8-9]. It is not necessary that the effect of pesticide on human health applicable directly but some pesticides accumulate into the food to a toxic level[10-11].

Several investigators have studied the side effects of the pesticides on heredity material of different plant cells[12]. Some of the pesticides adversely affect the genetic system by producing various types of chromosomal abnormalities[13]. One such pesticide is chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridylphosphorothioate), a broad-spectrum insecticide used for pest control in a wide variety of crop and animal husbandry applications[14]. The other pesticide used in our experiment is Endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9ahexahydro-6, 9-methano-2, 4, 3,-benzodioxathiepine 3-oxide,) which is also a broad spectrum contact insecticide belonging to chlorinated cyclodiene group is in use to control this pest for long time. It is alsorecommended to control insects like pod borer and aphids under Integrated Pest Management (IPM) package[15].

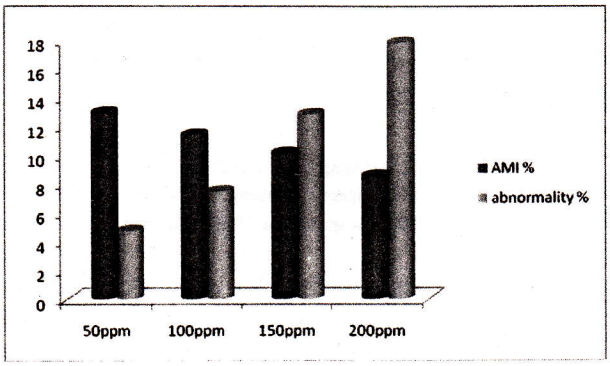
Since there is no significant work done the effect of these pesticides i.e. Endosulfan and Chlorpyrifos on mitotic cell division of *Vigna unguiculata.* Hence, the present study was aimed to detect the comparative effect of Endosulfan and Chlorpyrifos on mitotic cell division and somatic chromosome of *Vigna unguiculata.*

Material and Methods

For mitotic studies, seeds of *Vigna unguiculata* var. K 5269 were obtained from Chandrasekhar Azad Agricultural University, Kanpur, India. The seeds of cowpea were soaked in water for overnight. The soaked seeds were then put on wet petridishes for 2-3 days for germination. The germinated seeds were treated with different concentrations of Endosulfan and Chlorpyrifos (50 100 150 and 200ppm of each) for 5h before fixation time. The root tips were cut carefully and fixed in acetoalchol solution (1 acetic acid: 3 absolute alcohol) for 24 hours. The root tips were hydrolysed in 1N HCl for 5 minutes and squashed in 2%acetocamine for cytological studies. The active mitotic index and abnormality percentage were calculated.



Graph 1. Mitotic index and abnormality % induced by different concentrations of Endosulfan.



Graph 2. Mitotic index and abnormality % induced by different concentrations of Chlorpyrifos.

Table 1. Various types of mitotic anomalies and active mitotic indices (AMI) as induced by treatment of endosulfan and chlorpyrifos.

Pesticide	Treatment doses	Metaphasic abnormality %					Anaphasic abnormality %					Total ab%	AMI %
		St	Cm	Pr	Un	Ot	St	Lg	Br	Un	Ot		
	Control	-	-	-	-	-	-	-	-	-	-	-	13.37
Endosulfan	50ppm	0.27	0.64	0.60	0.56	0.68	0.56	0.60	0.32	0.28	0.75	5.26	12.54
	100ppm	0.54	0.95	0.78	0.68	1.18	0.72	0.86	0.69	0.57	1.35	8.33	11.38
	150ppm	1.26	1.64	1.45	1.06	1.78	1.22	1.64	1.02	0.86	1.86	13.79	9.09
	200ppm	1.87	2.48	2.08	1.56	2.65	1.58	2.10	1.52	1.48	2.25	19.57	7.42
Chlorpyrifos	50ppm	0.30	0.62	0.54	0.45	0.60	0.51	0.60	0.36	-	0.78	4.76	12.96
	100ppm	0.48	0.89	0.78	0.68	1.15	0.68	0.92	0.55	0.31	1.06	7.50	11.43
	150ppm	1.18	1.85	1.26	0.98	1.76	1.08	1.54	0.87	0.72	1.62	12.86	10.17
	200ppm	1.58	2.15	1.75	1.45	2.55	1.54	2.05	1.36	1.33	2.10	17.86	8.59

Abbreviations- St-stickiness, Cm- C metaphase, Pr-precocious movement, Un-unorientation, Lg-laggard, Br-bridges, ot-other abnormalities, ab- abnormality.

Results and Discussion

The effect of Endosulfan and Chlorpyrifos on mitotic index and abnormality percentage have been given in Table 1. The result shows that the increase in concentration of pesticides decreases the mitotic index and increases the chromosomal abnormality (Table 1). The mitotic index value for control was found to be highest (13.37%). No abnormality was recorded in control slides. The heighest value of abnormality percentage was recorded at 200 ppm treatment of Endosulfan (19.57%). However, lowest percentage of abnormality was recorded at 50ppm concentration of Chlorpyrifos (4.76%). On comparison of Endosulfan and Chloropyrifos, the former was proved to be more deterimental than later.

The different type of chromosomal abberations was presented in Fig. 1. The most common type of abnormality observed, is precocious arms at metaphase (Fig.1c) and lagging chromosome at anaphase (Fig.1g). Among other metaphasic abnormalities unorientation (Fig.1e) ring formation (Fig. 1h), C- metaphase (Fig. 1f) and disturbed metaphase (Fig.1i) were also frequently reported. The most frequent anaphasic abnormality was

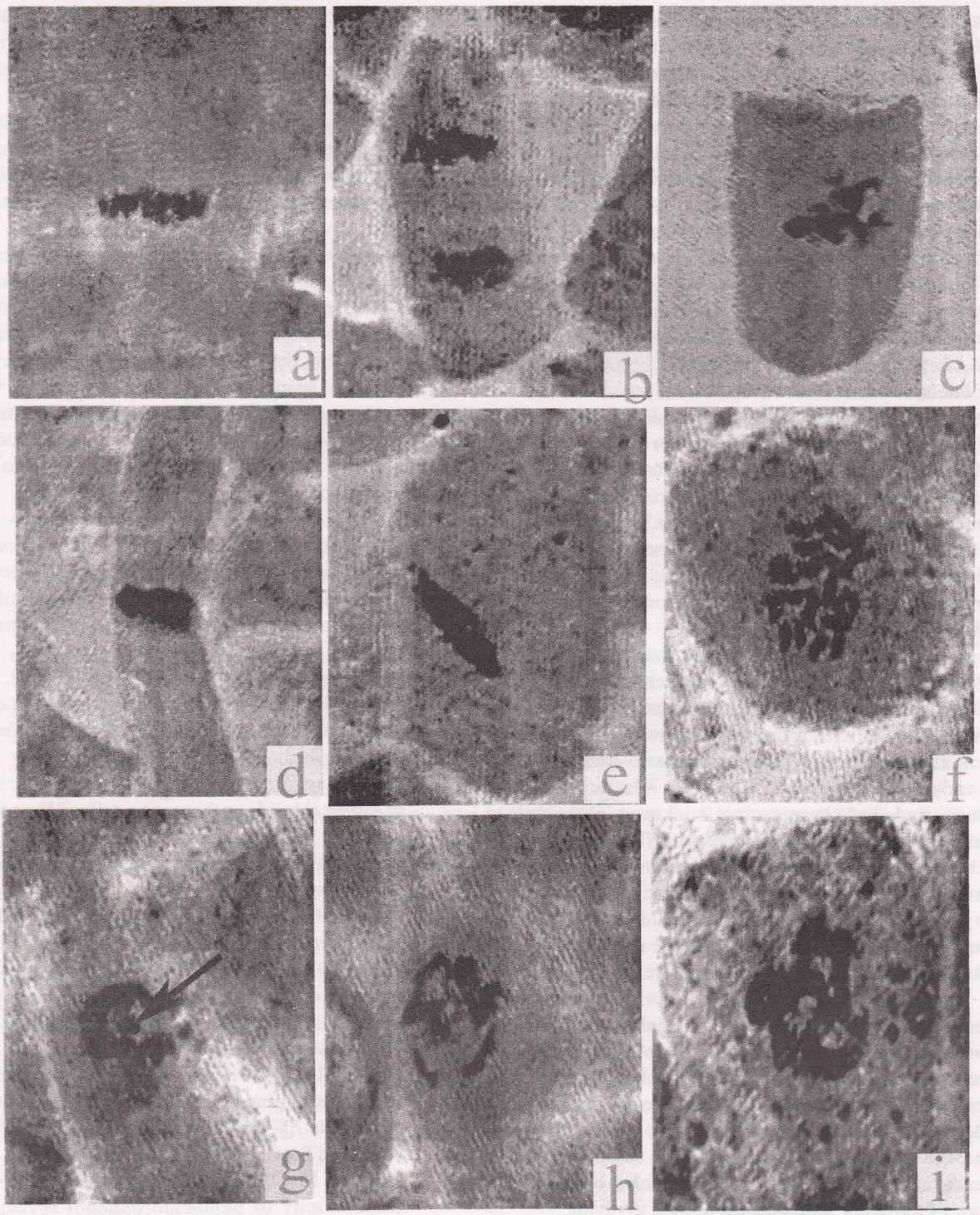


Fig.1. a-i. a) Normal metaphase; b)Normal anaphase; c) Precocious arms at metaphase; d) Sticky metaphase; e) Unorientation with sticky chromosome ; f) C metaphase; g) Laggard at early anaphase; h) Ring formation at metaphase; i) Disturbed metaphase.

laggard stage of chromosome followed by stickness (Fig.1g) and bridges.

The pesticides have broad spectrum of activities with long residual effects and wide use in agriculture and horticulture. The studies about the effect of different pesticides found out some of the genotoxic effects on plants[16]. The changes on mitotic activities like mitotic index, phase indices and induction of chromosomal abnormalities appeared in varying degrees depending on the dose treatment of Endosulfan and Chloropyrifos (Table 1).The prominent metaphasic anomaly observed is C metaphase. According to Nagl[17] the treatment of insecticides to the roots caused blockage of the cell cycle at metaphase which subsequently resulted into C-metaphase. However, according to Inceer *et al.*[13] reported that C - metaphase was produced as a result of the inhibition of spindle fiber formation (Fig.1f) Precocious arms and precocious chromosomes formation could be caused by stickiness of chromosomes[18]. Other interesting abnormalities were bridges, sticky bridges and chromosomal bridges which were observed frequently in the different types of treatment doses. This could be due to general stickiness of chromosome breakage and reunion[19-20].

Another frequent anomaly observed at metaphase and anaphase was the sticky nature of chromosomes which could be due to delay in chromosomal movement. As a result, the chromosomes could not reach the poles and remained scattered in the cytoplasm and appeared condensed and sticky[21]. However Klasterska *et al.*[22] suggested that chromosomal stickiness arose due to improper folding of chromosome fibers into single chromatid and thus there is an intermingling of fibers, making chromosomes to become attached to each other by means of subchromatid bridges. However, unorientation of chromosome may be due to the spindle formation or the destruction of spindle fibers formed[23].

The changes of mitotic activity in plants have been attributed to many factors. The inhibition of mitotic index may be due to the interference of ethion in the normal process of mitosis by reducing the number of the dividing cell[20,24]. Many other investigations were attributed to depression of mitotic activity due to the inhibition of protein synthesis[25]. Mitotic inhibition could also be due to the inhibition of DNA synthesis which is considered as one of the major prerequisites for cell to divide[20].

In our report, the mitotic index decreased alongwith increasing concentrations and durations of treatment of endosulfan and chlorpyripfos (Table 1). This result suggests that endosulfan and chlorpyripfos caused cytological changes and induced a wide range of mitotic abnormalities in the root tip cells of cowpea. Similar results were obtained after treating A. cepa root cells with insecticides and pesticides[26-27].

The present observation revealed that the pesticide exerts a mitodepressive effect upon the root tip cells of cowpea. It has capability of producing variety of mutants, chromosomal aberrations and toxic effects in the long run, even below the recommended dose. Such chromosomal abnormalities can affect the vigor, fertility, yield and resistance to the pathogens. In conclusion this result indicates that pesticides like Endosulfan and Chloropyrifos are absorbed by heigher plants and may affect adversely the genetic system of the plants. Wide application of pesticides in agricultural practices would cause detrimental effect on genfetic constitution of economically important vegetable crop like cowpea and others. Hence there is need of testing the genotoxic effect of pesticides on plants.

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