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EVALUATION OF CYTOGENETIC HAZARDS OF TWO PESTICIDES (ROGOR AND BAVISTIN) AND AN ANTIBIOTIC (STREPTOMYCIN) IN MEIOTIC CELLS OF *LENS CULINARIS*

UPENDRA KUMAR*
Department of Botany, Acharya Narendra Deva College, Shahpur Patory (Samastipur)-848 504
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This paper deals with the evaluation of the effects of two pesticides Rogor and Bavistin and an antibiotic Streptomycin on cytological behaviour in meiotic cells of *Lens culinaris*. Studies were made through seed-soak treatment. Pre-soaked seeds were treated with different concentrations of chemicals and after through washing with distilled water, they were allowed to grow in experimental plots. Meiotic abnormalities such as multivalents, univalents, laggards, bridges/fragments and micronuclei were induced by the mutagenic treatments. As a result of these aberrations, the tetrad formation was highly irregular and caused a high polen sterility. Chromosomal aberrations and pollen sterility were dose-dependent. Streptomycin appeared to be the most potent clastogenic agent followed by Rogor and Bavistin. The results obtained establish the cytotoxic effects of these chemicals.

Key Words : Chemicals (Pesticides and antibiiotic), *Lens culinaris*, cytological abnormalities.

In India, as in many other countries, the recent years have seen a large scale application of pesticides and antibiotics in almost all the fields. The use of such chemicals has become an indispensable requirement for the control of various diseases but their frequent indiscriminate use may have many undersirable secondary consequences. The side-effects of these chemicals and their residues on treated crops and other plants, particularly their effects on cellular system and chromosomes are not clearly understood.

The present communication deals with the assessment of the effects of two pesticides Rogor and Bavistin and an antibiotic Streptomycin on meiotic behaviour in *Lens culinaris*. These chemicals were chosen because they are widely used in India against harmful insects, fungi and bacteria. *Lens culinaris* was selected as an inexpensive testing system for the mutagenic effects of these chemicals in higher plants.

MATERIALS AND METHODS

Two pesticides Rogor (0-0-dimethyl N-methyl carbamidomethyl-dithiophosphate) and Bavistin (2-methyl benzimidazole carbamate) and an antibiotic Streptomycin (N-methyl-L-glucosamidinostreptosidostreptidine) of four varying concentrations each (0.10, 0.25, 0.50 and 1.00%) were selected. Dormant and genetically pure seeds of *Lens culinaris*, utilized in the present investigation, were procured from the Division of Genetics, I.A.R.I. new Delhi. The seeds were first soaked in water for 12 hours and then treated with freshly prepared solutions of different concentrations of the used chemicals for 6 hours. All the solutions were adjusted to pH 7.0 at room temperature of 21°C±2°C with intermittent shaking.

After treatment, the seeds were thoroughly washed in water and 200 seeds of each treatment were sown to properly managed experimental field according to the randomized block design. Alongwith each treatment, one corresponding control was also maintained. Special care was taken to provide uniform field conditions for the plants till harvest.

In order to analyse the chromosomal behaviour in meiotic system of the treated plants. The floral buds of different sizes were fixed in aceto-alcohol (1:3) and cytological preparations were made in 2% aceto-carmine smears of PMCs. A minimum of 500 PMCs per treatment were analyzed to score the chromosomal anomalies. Stainability of pollen grains with acetocarmine was used as the index for determining pollen sterility.

RESULTS

The study of chromosome behaviour during meiosis reflects the internal environment of the

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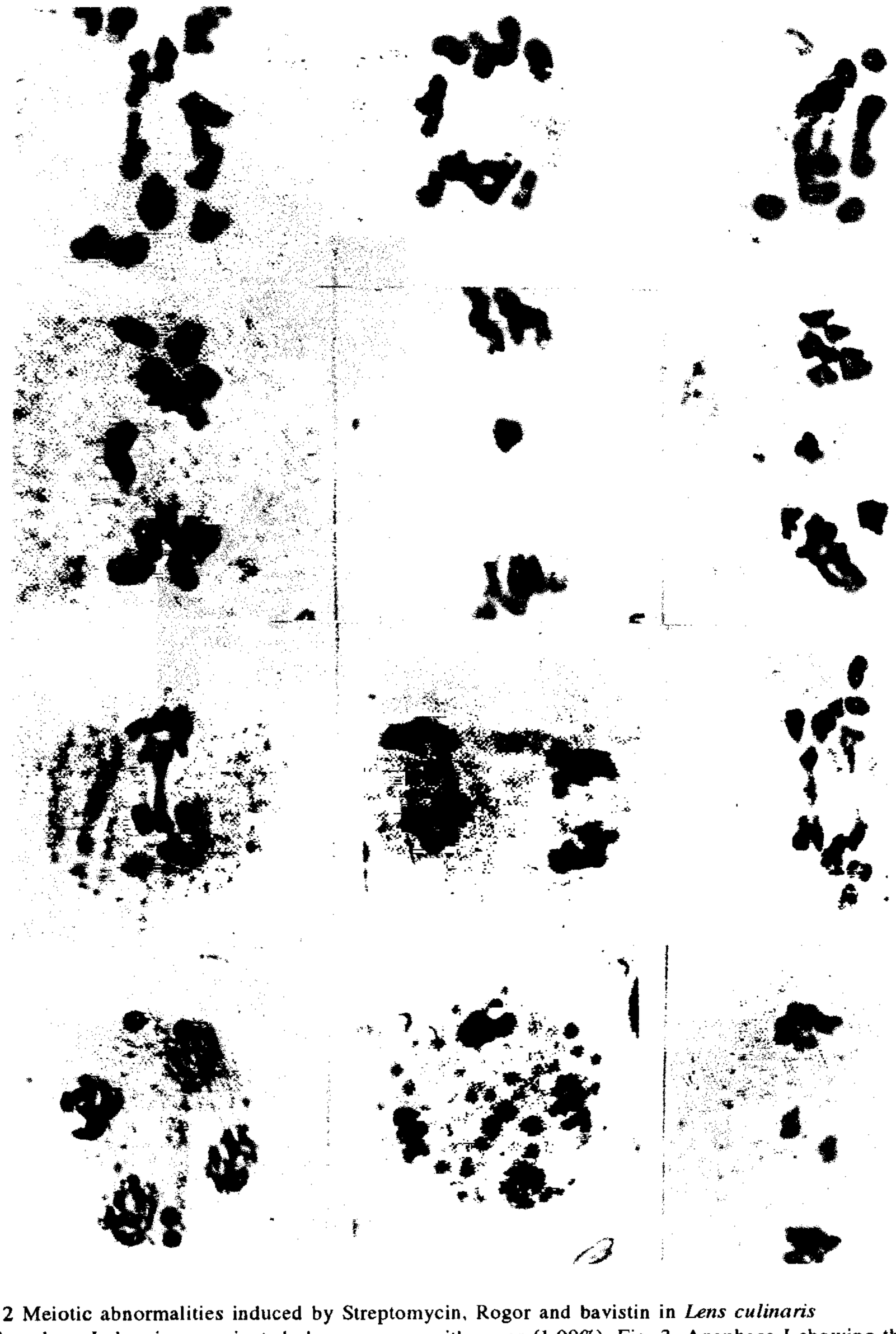
*Address for correspondence : Manas Marg, House No. 171, South Nehru Nagar, Patliputra, Patna-800 013.



Figures 1-12 Meiotic abnormalities induced by Streptomycin, Rogor and bavistin in *Lens culinaris*
Figs. 1-2. Anaphase I showing unoriented chromosomes with rogor (1.00%). Fig. 3. Anaphase I showing three bridges with rogor (1.00%). Fig. 4 Anaphase I showing a lagging bivalent with streptomycin (0.50%). Figs. 5-6. Anaphase I showing laggards with rogor (0.25%). Fig. 7. Anaphase I showing single bridge with bavistin (0.25%). Fig. 8. Anaphase II showing single bridge with streptomycin (0.50%). Fig. 9. Anaphase I showing double bridges with rogor (1.00%). Fig. 10. Telophase II showing three micronuclei with streptomycin (1.00%). Fig. 11. Telophase II showing multiple number of micronuclei with rogor (1.00%). Fig. 12. Anaphase I showing laggards with bavistin (1.00%).

plant and contributes to an understanding of various cytological phenomena. The results recorded from the cytological studies of the PMCs from the chemical treated materials are summarised in Tables la and b. It was observed at diakinesis that the average frequency of chiasmata decreased with the

Table 1(a): Percentage of different types of abnormalities occurring in different meiotie stages of *Len culinaris* as affected by chemicals.

Treatment (%)		Total No. of PMCs	Total No. of abnor-mal cells	% of aberrant PMCs	% of abnormalities at metaphase I				% of abnormalities at anaphase/telophase I		
					Sticki-ness	Uni-valents	Multi-valents	UC	Bridges	Laggards	Bridges with laggards
Control		570	-	-	-	-	-	-	-	-	-
S-	0.10	568	16	2.79	0.88	0.21	-	0.34	0.39	0.15	-
	0.25	581	39	6.75*	1.94	0.36	0.12	0.74	0.88	0.50	0.28
	0.50	588	68	11.56*	2.85	0.91	0.42	0.90	1.30	1.07	0.49
	1.00	563	98	17.41*	3.96	1.24	0.93	1.04	2.55	2.00	0.83
R-	0.10	570	13	2.29	0.84	0.14	-	0.25	0.23	0.12	-
	0.25	589	35	5.87*	1.85	0.31	0.10	0.51	0.78	0.40	0.18
	0.50	567	52	9.16*	2.44	0.68	0.33	0.76	1.13	0.82	0.31
	1.00	599	94	15.71*	3.68	1.14	0.85	0.89	2.37	1.72	0.64
B-	0.10	606	13	2.14	0.78	0.11	-	0.23	0.22	0.12	-
	0.25	591	31	5.18	1.69	0.27	0.09	0.64	0.65	0.32	0.09
	0.50	587	51	8.70*	2.40	0.64	0.27	0.74	1.05	0.78	0.26
	1.00	600	83	13.76*	3.41	0.98	0.76	0.82	2.16	1.62	0.41

* Statistically significant at $P<0.05$ in 2 x x X^2 contingency test.
S = Streptomycin; R = Rogor; B = Bavistin; UC = Unoriented chromosomes

Table 1(b): Anormalities at different stages of meiosis II (Aberrations in percentage).

Treatment (%)		Metaphase II UC	Anaphase/Telophase II			Tetrad abnor-malities	Pollen sterility
			Bridges	Laggards	bridges with laggards		
Control		-	-	-	-	-	-
S-	0.10	-	0.34	0.19	-	0.29	6.10
	0.25	0.16	0.57	0.48	0.12	0.60	9.36
	0.50	0.31	0.83	0.90	0.32	1.26	11.25
	1.00	0.54	1.14	1.09	0.56	1.53	14.00
R-	0.10	-	0.28	0.18	-	0.25	5.87
	0.25	0.12	0.52	0.42	0.09	0.59	8.76
	0.50	0.15	0.70	0.68	0.23	0.89	10.41
	1.00	0.49	1.06	0.97	0.45	1.45	12.60
B-	0.10	-	0.26	0.17	-	0.25	5.72
	0.25	0.10	0.38	0.38	0.07	0.50	8.06
	0.50	0.16	0.70	0.66	0.21	0.83	9.28
	1.00	0.34	0.91	0.80	0.33	1.22	11.15

S = Streptomycin; R = Rogor; B = Bavistin; UC = Unoriented chromosomes.

increase in concentration. It was because of an early separation of the chromosomes of a bivalent. In most of cells, bivalents were associated with varying number of univalents and multivalents with ring and chain types at diakinesis and metaphase I. Rod bivalents were of common occurrence. Studies of later stages revealed that the treatment with the chemicals resulted in various types of clastogenic changes such as stickiness and clumping, laggards, bridges and micronuclei (Fig. 1-12) with varying frequencies (Tables la, b).

Stickiness of chromosomes seemed to be a common effect caused by all the experimental agents. The PMCs carrying bridges were observed both at anaphase I and II stages in considerable percentage (Figs. 3, 7, 8, 9). However, the frequency was less at anaphase II where it was 1.1%, 1.06%, and 0.91%, in contrast to 2.55%, 2.37% and 2.16% during anaphase I in the materials treated with highest (1.00%) concentration of the chemicals. The unoriented (Figs. 1, 2) and lagging chromosomes (Figs. 4, 5, 6, 12) at various divisional stages

of PMCs were resulted in the formation of micronuclei (Fig. 10,11) at telophase II and these micronuclei varied in size and number from cell to cell.

Apart from different types of chromosomal irregularities, abnormal cytokinesis was fairly frequent and abnormal sporads such as triads and polyads were noticed. Pollen grains were variable in size. The plants showed a high pollen sterility as a result of these anomalies. A definite correlation between chromosomal damage and pollen sterility was recorded.

As is apparent form Tables la and b, chromosomal irregularities and pollen sterilities were dose-dependent in case of all the three chemicals under experiment.

The data were subjected to the relevant statistical analysis. The 2 x 2 contigency X^2 analysis revealed that the incidence of aberrant cells was higher in Streptomycin treatments than in pesticide (Rogor and Bavistin) treatments.

DISCUSSION

The induction of cytological disturbances in the treated meiotic cells is of utmost value as it results in genetic damage that is faithfully handed over to the next generation. The frequency and types of anomalies recorded in the present study clearly indicated that all the three chemicals used here were capable of inducing deleterious effects on chromosomes of *Lens culinaris*. The results also showed that there existed a linearity between the concentration and the amount of aberrations. The gross abnormalities suggested a similarity to the results recorded by Wuu and Grant 91967) with atrazine and others, Amer and Farah (1976, 1983) with sevin, duphar and rogor, Reddy and Rao (1982) with dimecron, rogor and planofix, Kumar and Sinha (1991) with rogor, bavistin and streptomycin.

The presence of univalents at metaphase I suggests that following chemical treatments, chromosomes display a weak pairing resulting into complete or partial failure of synapsis between homologous chromosomes. The occurrence of such alteration was in line with some earlier reports obtained on *Vicia faba* following treatment with some pesticides (Singh *et al.*, 1978; Amer and Farah, 1980). The formation of multivalents was the possible outcome of exchange between non-homologous chromosomes. The presence of stickiness in majority of cells supported the idea of Amer and Ali (1983) that chromosome stickiness is a general effect induced in PMCs after treatment with different chemical agents. it could be possible that stickiness originates as a consequence of disturbances in the cytochemically balanced reaction by the effect of chemical treatment. Darlington (1953) reported that stickiness arises from the depolymerisation of DNA on the surface of chromosomes. However, stickiness appeared to be a temporary effect in the present study since its percentage decreased gradually from the early stages of the first division to the late stages of second division. This suggested either the abnormality has reverted back to normal or such PMCs did not complete their meiotic cycle (Jain and Sarbhoy, 1988). The retardation of chromosome movement resulting in lagging chromosomes seemed probably due to the fact that treatment produced hindrance in the movement and stickiness of the bivalents. Such phenomenon was also recorded in *Vicia faba* after treatment with 2,4,5-trichlorophenol (Amer and Ali, 1974).

Other significant cytological aberrations like bridges and micronuclei have been frequently observed in a wide range of plants with the application of other pesticides and antibiotics. it is well known that bridges lead to structural changes in the chromosomes. Formation of micronuclei at telophase II in the present study might have resulted from laggards of anaphase I and II (Liang *et al.*, 1967; Amer and Ali, 1974). it appeared that micronuclei are resultant of clastogenic events of the cell rather than motoclastic events (Sparrow and Singleton, 1953).

The occurrence of abnormal sporads in the present investigation can be traced to errors in the cleavage of cytoplasm or due to spindle abnormalities. There was a definite correlation between chromosomal damage and pollen sterility and a similar observation has been made in *Capsicum* by Rajam and Subhash (1984). PMCs with aforementioned abnormalities may terminate in genetic breakdown or make them less competitive than normal ones limiting their transmission to the subsequent generation (Liang *et al.*, 1969). Thus, the disturbances

in normal chromosome behaviour in PMCs will surely effect the pollen fertility. The results clearly indicated that pollen fertility decreased with the increase in treatment.

From the above results it can hence be concluded that all the three chemicals tested here were effective and possessed strong mutagenic activity with radiomimetic action. The differential mutagenic activities of these chemicals were possibly due to differences in their chemical nature and potent ingredients. Chemicals carrying such actions may alter the genetic constitution of the crop plants (Wuu and Grant, 1967; Ma 1981). In fact, the subsequent long-term effects of these chemicals on the plant genome were not carried out. The present study, hence, does not demand a ban on the application of these chemicals because of their benefits. However, it may safely be inferred that one should be cautious in identifying the disease in preliminary stages so as to control with less concentrations of the chemicals and it is also important that the use of these chemicals in high concentration should be avoided to safeguard the genetic purity of the crop plants.

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