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Short Communication

Pesticide induced genotoxicity in legumes

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Mitotic studies were carried out in three selected pulses *viz.*, mungbean [*Vigna radiata* (2n = 22)], urdbean [*Vigna mungo* (2n = 22)] and chickpea [*Cicer arietinum* (2n = 16)] to score the genotoxicity. Seeds obtained from Indian Institute of Pulses Research [ICAR], Kanpur, were presoaked in distilled water for 16 hours under laboratory conditions and treated with various concentrations of aldrin for 4 hours. The solutions of different concentrations were freshly prepared in phosphate buffer and adjusted to pH-7. After the treatment, the seeds were thoroughly washed in running water and were placed on a moist filter paper in petri-dishes for germination. The root-tips were fixed in freshly prepared 1:3 mixture of acetic acid and ethanol for 24 hours. Thereafter the tips were stored in 70% alcohol in a refrigerator. Root tips were stained in 2% acetocarmine stain following usual hydrolysis method. Cytological examination was done on 4-5 slides from each treatment. The resulting data shows the mean percentage frequencies of abnormalities. Mitotic indices were calculated for all the doses.

Mitosis was normal in root tips under control condition. However, in root tips of treated seeds there was a gradual reduction in mitotic index in all the three pulses as presented in Table 1. Treatment with all the five concentrations of the aldrin, not only reduced the frequency of dividing cells but a wide spectrum of chromosomal abnormalities was also recorded in the treated roots. The individual abnormalities and the total abnormal cells increased alongwith the increase in concentrations of aldrin, in all the three legumes. Earlier reports also showed that the pesticides can cause chromosomal aberrations during the cell division [1, 2]. A maximum frequency of 2.71% of aberrant root tip cells was noticed at 0.5% treatment in mungbean. The major cytological abnormalities detected were stickines fragments, laggards, chromatin bridges, binucleate cells and micronuclei (Fig. 1-12).

In the treated roots, a gradual reduction in mitotic index was noted with the increase in concentration. In all the three legume, the chromosomal fragments were observed at all the concentrations except at 0.1% in urdbean. Highest frequency (0.45%) of fragments was observed at 0.5% treatment in mungbean. Fragments might have arisen due to the stickiness of the chromosomes and consequent failure of arrival of chromatids to poles. Fragments may also be acentric chromosomes which are formed as a result of inversion [3].

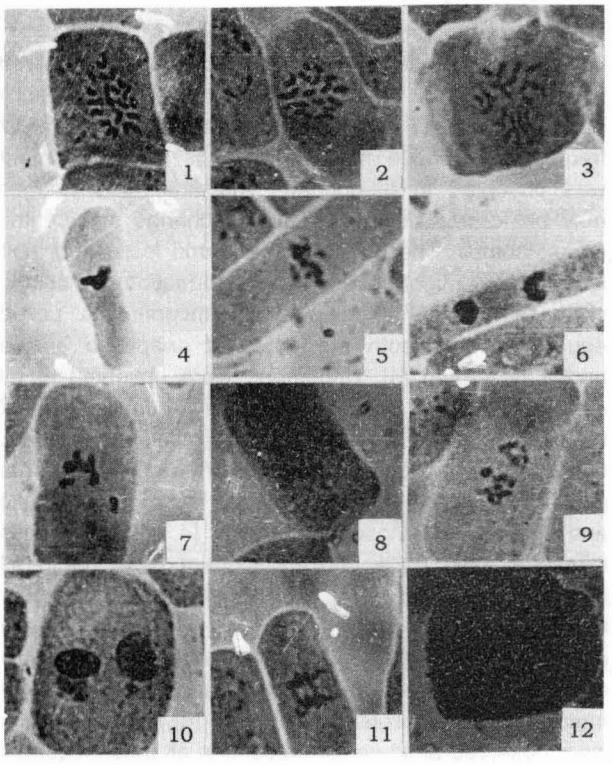
The chromatin bridges were commonly observed at anaphase at all the concentrations. Highest frequency of bridge (0.83%) at 0.5% aldrin treatment and lowest frequency of bridge (0.23%) at 0.1% aldrin treatment was observed in mungbean. The anaphasic bridges may be formed due to unequal exchange or dicentric chromosomes. The breaks at the same locus and their lateral fusion might have led to the formation of dicentric chromosomes. The dicentric chromosome is pulled equally towards both the poles at anaphase and a bridge is formed [4]. Breakages were observed at all concentrations of aldrin in all three legumes. Highest frequency (0.48%) of breakage was observed at 0.4% treatment in mungbean. A number of organophosphorus pesticides have been reported to be a radiomimetic and induce chromosomal breaks [4, 5]. Stickiness increased upto 0.2% concentration of pesticide in all these legumes and decreased at higher concentrations. Chromosome stickiness leading to sticky metaphase and precocious separation of chromosomes are possibly due to the effect of chemicals in breaking the protein moiety of nucleoprotein backbone [6]. The laggards were observed at all concentrations in all these legumes but they did not show any specific pattern. Highest percentage (0.56%) of lagging chromosomes was observed at 0.4% concentration in mungbean and 0.5% concentration in chickpea.

Another significant observation noticed, was the formation of binucleate cells indicating that the pesticides inhibited the cell plate formation. In these legumes, binucleate cells were seen at all the concentrations except 0.1% concentration but the exact chromosome number of the binucleate cells could not be established because of condensation. The highest percentage of binucleate cells was observed during the treatment of 0.5% concentration of aldrin in chickpea.

Table 1. Percentage abnormalities and mitotic index in root tips of mungbean, urdbean and chickpea after treatment with Aldrin

Treatment%	mungbean (*Vigna radiata*)									urdbean (*Vigna mungo*)									chickpea (*Cicer arietinum*)								
	ChFr %	CHBr %	Break %	ST %	LgCh %	DNC %	MI	RDR	RAR	ChFr %	ChBr %	Break %	ST %	LgCh %	DNC %	MI	RDR	RAR	ChFr %	ChBr %	Break %	ST %	LgCh %	DNC %	MI	RDR	RAR
Control							18.24	-	-							16.27	-	-							17.39	-	-
Aldrin																											
0.1	0.16	0.23	0.31	0.39	0.39	-	10.84	–9.05	1.48	-	0.29	0.14	0.21	0.29	-	10.26	–7.18	0.93	0.07	0.27	0.33	0.40	0.27	-	10.58	–8.24	1.34
0.2	0.23	0.31	0.38	0.46	0.31	0.08	8.63	–11.75	1.77	0.07	0.38	0.23	0.30	0.38	0.07	9.20	–8.44	1.43	0.14	0.35	0.28	0.42	0.42	0.07	9.47	–9.59	1.68
0.3	0.30	0.53	0.30	0.38	0.46	0.15	6.53	–14.32	212	0.15	0.44	0.29	0.22	0.29	0.15	7.18	–10.86	1.54	0.20	0.41	0.27	0.34	0.48	0.14	6.69	–12.95	1.84
0.4	0.32	0.64	0.48	0.32	0.56	0.16	5.76	–15.26	2.48	0.23	0.62	0.23	0.16	0.54	0.08	6.44	–11.74	1.86	0.29	0.65	0.36	0.29	0.51	0.22	5.72	–14.13	2.32
0.5	0.45	0.83	0.45	0.22	0.53	0.23	4.74	–16.51	271	0.28	0.64	0.28	0.14	0.50	0.14	5.31	–13.09	1.98	0.35	0.71	0.35	0.21	0.56	0.28	5.42	–14.49	2.46
Coefficient of Correlation between treatment and various chromosomal abnormalities																											
	0.977*	0.994*	0.760	0.848	0.818	0.966*				0.998*	0.975*	0.748	0.723	0.789	0.749				0.994*	0.969*	0.499	0.931	0.938*	1.000			

ChFr-Chromatin fragments, ChBr - Chromatin bridges, Break - breakage, ST - Stickiness, LgCh - Lagging chromosomes, DNC - Dinucleate Cells, MI - Mitotic index, RDR - Relative division rate, RAR - Relative abnormality rate
*Significant at 5% level



Figs. 1. Metaphase of mungbean [*V. radiata* (2n = 22)]; 2. Metaphase of chickpea [*C. arietinum* (2n = 16)]; 3. Metaphase of urdbean [*V. mungo* (2n = 22)]; 4. Stickiness at metaphase in mungbean; 5. Metaphase in chickpea showing fragmentation; 6. Stickiness at anaphase in urdbean; 7. Laggards at metaphase in mungbean; 8. Anaphase bridge in chickpea; 9. Unequal segregation and lagging chromosomes at anaphase in urdbean; 10. Binucleate cell in mungbean; 11. Anaphase showing double bridge in chickpea; 12. Micronuclei in urdbean

The present results clearly show that the pesticide (aldrin) caused genotoxicity in the root meristems of legumes and the continuous application of the pesticide may alter the genetic constitution of crop plants. There was a linear correlation between the different concentrations of pesticide and the percentage of abnormalities (Table 1). The coefficient of correlation shows that the percentage of chromosomal fragments (ChFr) and chromosomal bridges (ChBr) was found significantly different at 5% level in each of the three legumes. In addition to it, the percentage of dinucleate cells (DNC) in mungbean and the percentage of lagging chromosomes (LgCh) in chickpea was also significantly different with the treatment at 5% level. This in turn suggests that the effect of aldrin doses is relatively more pronounced in the above mentioned chromosomal abnormalities rather than breakage (Break) and stickiness (ST). Thus, it can be concluded that aldrin, which is frequenly used like other pesticides in agriculture has positive chromotoxic effects. Present investigation warns against indiscriminate spraying of this chemical.

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