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CYTOMORPHOLOGICAL CHANGES INDUCED IN BREADWHEAT FOLLOWING SEED TREATMENT WITH PESTICIDES AND MUTAGENIC CHEMICALS

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ABSTRACT

Dry healthy and uniform seeds of breadwheat (*Triticum aestivum* L.) cv. K 68 were treated for 3 h with 0.1% solution of the active ingredients of four commercial insecticides: Ekalux, Anthio, Heptachlor and Gammaxene; three fungicides: Diathane M-45 (DM-45), Diathane Z-78 (DZ-78) and Bavistin; and two known mutagenic chemicals: dimethyl sulphonate (DMS) and ethylmethane sulphonate (EMS). All the treatments reduced germination percentage, except Anthio, which showed a significant increase over the control. Various treatments significantly affected seedling height on 20th day, except DM-45 and Heptachlor. The dry matter of 20-day-old seedlings and number of spikes did not show any significant difference, however, some of the treatments showed increase over the control. Pollen fertility was adversely affected by most treatments, except Anthio and EMS, which showed a significant increase. Several cytological anomalies, such as, bridges, fragments and laggards, were observed with higher frequency at mitotic anaphase and meiotic anaphase I stages.

Key words: Cytological, morphological changes, seed treatment, pesticides, mutagens, breadwheat.

In the earlier investigations, the application of pesticides has been reckoned for bringing changes in morphological parameters, viz., reduction in germination and seedling height [1–3] and pollen fertility [2, 4]. Besides, the pesticides are known to induce many cytological aberrations in plants leading to mitotic configurations [1–3, 5, 6] and meiotic anomalies [2, 4, 6–9]. Some pesticides are also known for their mutagenic effects [5, 10–12]. However, only a few pesticides have been found not to induce cytological abnormalities [3]. Nevertheless, the frequency of aberrations induced in the pesticides treated barley seeds was many times greater than the seeds treated with growth regulators [13].

It is important that a rigorous testing for the mutagenic effect of pesticides is undertaken in view of their widespread use and the seriousness of the hazardous consequences. It is generally observed that the mutagenic chemicals induce chromosomal damages [14]; mitotic and meiotic aberrations have often been employed for quick screening of mutagenic and carcinogenic chemicals.

The present investigation has been undertaken to investigate the effect of some pesticides and mutagenic chemicals on germination, early seedling vigour, spike number, dry matter production, pollen fertility and cytological aberrations in bread-wheat.

MATERIALS AND METHODS

Dry, healthy and uniform seeds of breadwheat (*Triticum aestivum* L.) cv. K 68 presoaked in distilled water for 1 h were treated with 0.1% solution of individual pesticides or mutagenic chemicals for 3 h and washed in running water for 2 h. The insecticides were: Ekalux [0,0-diethyl-0-(quinoxalinyl-(2)-thionophosphate], Gammaxene (1,2,3,4,5,6-hexachlorocyclohexane; $C_6H_6Cl_6$), Heptachlor (1,4,5,6,7,8, 8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methano indane; $C_{10}H_5Cl_7$) and Anthio [0, 0-dimethyl-S-(N-methyl-N-formoylcarbamoyl-methyl)-dithiophosphate]; and fungicides: DM-45 (manganese ethyl bisdithiocarbamate), DZ-78 (zinc ethylene bisdithiocarbamate) and Bavistin [2(methoxy-carbomoyl benzimedezol)]. Two potent mutagenic chemicals, ethylmethane sulphonate (EMS) and dimethyl sulphonate (DMS) were employed separately.

Four hundred seeds were used in each treatment, of which 100 were placed in the Petri dishes and remaining 300 seeds were sown in the field in randomized block design with three replications.

Germination was recorded on 3rd day. The height of 20-day-old seedlings in the Petri dishes was measured and their dry matter content determined.

In the field sown material, pollen sterility was scored in 10 randomly selected central spikelets from each treatment. The pollen grains were stained with 1% KI solution enriched with iodine crystals; the deep coloured pollen grains were treated as fertile. For each plant, about 300 pollen grains were observed. Effective (ear bearing) tillers and plant height were also recorded at maturity.

Cytological observations were made in root tips collected 72 h after seed soaking. Chromosomes were stained by the Feulgen technique and squash preparations were made in 45% acetic acid. Ten root tips per treatment and 10 cells per root were examined. Meiosis was analysed in 10 random spikes from each treatment, and 10 PMC per spike were scored for anaphase I (AI) anomalies. All the cytological materials were fixed in Carnoy's solutions (root tips in 3 ethanol : 1 acetic acid, and PMC in 6 ethanol : 3 chloroform : 1 acetic acid) and stored in 70% ethanol.

Observations on chlorophyll deficient M_1 plants were taken from seedling to flowering stage, and in M_2 at seedling stage, and classified into various categories according to Gustafsson [15]. The seeds from M_1 plants were sown as individual progenies.

Observations were recorded in both sets of treatments and the means analysed statistically according to complete randomised block design [16]. All the statistical tests were applied to the transformed data but the means are presented on the original scales.

RESULTS AND DISCUSSION

The various treatments exhibited significant difference (Table 1). The pesticides (except Anthio) and mutagenic chemicals generally caused reduction in germination. All the treatments, except DM-45 and Heptachlor, significantly reduced seedling height on 20th day, but none of the treatments showed significant difference for plant height at maturity. Further, none of the treatments exhibited significant

Table 1. Analysis of variance for various traits in wheat cv. K 68 treated with pesticides and chemical mutagens

Source of variation	d.f.	Mean squares							
		germination	20-day-old seedlings		height at maturity	spike number	pollen sterility	anomalies	
			height	dry matter				mitotic anaphase	meiotic anaphase I
Replications	2	0.1	0.5	0.01	166.3**	5.5	1.2	0.5	0.19
Treatments	9	61.5**	5.9**	0.33	33.7	2.1	150.4**	133.4**	97.26**
Error	18	1.1	0.3	25.02	30.8	2.2	1.4	1.0	1.09

**Significant at 1% level.

difference for dry matter yield of 20-day-old seedlings and spike number over the control (Table 2). However, pollen fertility generally improved as a result of treatments, except that the Anthio (80.7%) and EMS (83.0%) treatments caused a significant reduction in pollen fertility (Table 2). Thus, the present observations are in agreement with the earlier findings on germination and seedling height [1, 2, 17], pollen fertility [2, 4], and induced morphological changes [1, 5, 18, 19] in M_1 generation.

Table 2. Effect of seed treatment with pesticides and mutagens on germination, plant height, spike number, pollen sterility and cytological anomalies in K 68 wheat

Treatment	Germination (%)	20-day-old seedlings		Plant height at maturity, cm	Spike number, %	Pollen sterility, %	Abnormal mitotic anaphase, %	Abnormal meiotic anaphase I, %
		height, cm	dry matter, g					
Control	92.7	15.9	3.8	118.1	10.1	14.6	3.0	4.3
Ekalux	90.0	14.7	3.4	118.7	9.1	12.6	9.3	10.4
Anthio	95.3	14.9	3.3	117.9	10.9	19.3	11.3	11.3
Heptachlor	91.3	15.8	4.3	116.5	9.7	12.0	6.6	5.7
Gammaxene	87.0	13.1	3.8	122.1	9.7	8.3	4.0	4.7
DM-45	91.0	15.8	4.0	118.2	10.9	11.6	11.7	10.4
DZ-78	82.3	13.4	4.3	120.5	9.4	10.0	8.0	5.9
Bavistin	86.0	14.4	4.2	115.9	9.8	15.0	3.0	3.9
EMS	81.0	14.6	3.9	121.7	9.5	17.0	6.6	8.6
DMS	87.0	11.5	4.0	118.5	10.9	11.0	8.0	6.6
SE, ±	0.9	0.4	4.1	4.5	1.2	0.9	0.8	0.9
CD, 5%	1.8	0.9	8.6	9.5	2.5	1.9	1.7	1.8

The analysis of root tips and PMC revealed anomalies both at mitotic anaphase (Table 3) and meiotic anaphase I (Table 4). At mitotic anaphase, chromatin bridges were the most frequent aberration; bridges were more common than other aberrations. The total aberration frequency (including bridges) was maximum in DM-45 (11.7%) treatment, followed by Anthio (11.3%), Ekalux (9.3%), DZ-78 (8.0%) and DMS (8.0%) treatments. The maximum frequency of fragments was recorded following Anthio (2.7%) treatment, while laggards were maximum in the DM-45 (2.7%) treatment. The highest percentage of abnormal mitotic anaphase was recorded with DM-45 (11.7%). Convincingly, the pesticides Anthio and DM-45 showed significantly higher frequency of aberrations than the chemical mutagens tested, i.e., EMS and DMS (Table 3).

Table 3. Mitotic anaphase anomalies induced in wheat cv. K 68 pesticide and mutagenic treatments

Treatment	No. of cells observed	Mitotic anaphase anomalies, %			Total abnormal mitotic anaphases, %
		bridges	fragments	laggards	
Control	300	2.0	0.0	1.0	3.0
Ekalux	300	7.3	0.0	2.0	9.3
Anthio	300	7.3	2.7	1.3	11.3
Heptachlor	300	5.3	1.3	0.0	6.6
Gammaxene	300	4.0	0.0	0.0	4.0
DM-45	300	8.7	0.3	2.7	11.7
DZ-78	300	6.7	0.0	1.3	8.0
Bavistin	300	2.3	0.0	0.7	3.0
EMS	300	5.3	1.3	0.0	6.6
DMS	300	4.0	2.0	2.0	8.0

Pesticides are known to induce mitotic irregularities, especially anaphase bridges in many plant species [1, 2, 5, 19, 20]. The incidence of meiotic anomalies may be responsible for higher pollen sterility in the treated populations. At meiotic anaphase I (AI), laggards, bridges and fragments were observed in all the treatments (Table 4), but their frequency was significantly higher in the treatments of Anthio

Table 4. Meiotic anaphase I anomalies induced in wheat cv. K 68 by pesticide and mutagenic treatments

Treatment	No. of cells observed	Abnormal anaphase I configurations, %			Total abnormal anaphases, %
		bridges	fragments	laggards	
Control	300	2.3	1.3	0.7	4.3
Ekalux	300	4.7	2.7	3.0	10.4
Anthio	300	5.3	3.0	3.0	11.3
Heptachlor	300	4.7	0.7	0.3	5.7
Gammaxene	300	2.7	1.3	0.7	4.7
DM-45	300	5.0	2.7	2.7	10.4
DZ-78	300	5.3	0.3	0.3	5.9
Bavistin	300	3.3	0.3	0.3	3.9
EMS	300	5.3	1.3	2.0	8.6
DMS	300	4.0	1.3	1.3	6.6

(11.3%), Ekalux (10.4%), DM-45 (10.4%), and EMS (8.6%). At AI, bridges were generally more frequent in all the treatments, the frequency being highest (5.3%) equally with Anthio, DZ-78 and EMS. However, the frequency of fragments and laggards with DZ-78 (0.6%), Heptachlor (1.0%), and Bavistin (0.6%) was lower than even control (2.0%). On the other hand, the frequency of laggards, bridges and fragments was highest (11.3%) in the Anthio treated materials (Table 4).

Table 5. Chlorophyll mutation frequency and spectrum in M_2 generation of wheat cv. K 68 induced by pesticides and chemical mutagens

Treatment	No. of sedlings	Frequency of chlorophyll mutations, %					
		albino	xantha	chlorina	viridis	maculata	total
Control	1400	0.00	0.00	0.00	0.00	0.00	0.00
Ekalux	1390	0.14	0.29	0.36	0.22	0.07	1.08
Anthio	1300	0.23	0.31	0.46	0.38	0.15	1.53
Heptachlor	1368	0.07	0.07	0.15	0.15	0.07	0.51
Gammaxene	1388	0.07	0.00	0.07	0.07	0.07	0.28
DM-45	1380	0.29	0.36	0.22	0.14	0.14	1.15
DZ-78	1370	0.15	0.15	0.22	0.00	0.07	0.59
Bavistin	1366	0.22	0.07	0.07	0.07	0.00	0.43
EMS	1370	0.15	0.15	0.22	0.15	0.15	0.82
DMS	1372	0.07	0.22	0.15	0.15	0.15	0.74

Thus all the treatments induced cytological anomalies, but the pesticide treatments showed even greater frequency of aberrations than the established chemical mutagens like EMS and DMS. These treatments, expectedly, reduced germination percentage, seedling height and pollen fertility more effectively. Besides, chromosomal fragments and bridges would also lead to deficiencies and occasional duplications leading to mutations in the M_2 progeny [10, 18, 20, 21]. Analysis of chlorophyll mutations induced by pesticides and chemical mutagens (Table 5) revealed that Anthio (1.53%), DM-45 (1.15%), and Ekalux (1.08%) were more potent mutation inducing agents as compared to the known chemical mutagens like EMS (0.82%) and DMS (0.74%). In the recent past, cytotoxic effects leading to chromosomal damage and sister chromatid exchanges were reported for the herbicide maleic hydrazide [22]. Among all the treatments under comparison, Anthio, DM-45 and Ekalux produced a greater proportion of aberrant mitotic as well as meiotic anaphases and chlorophyll mutations than the remaining pesticides and mutagenic chemicals. Therefore, a rigorous testing for mutagenic effects of the pesticides before application is not only essential, but also a matter of serious concern, as frequent and excessive use of the pesticides may prove hazardous to the genetic constitution of crop plants.

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