DETECTION OF GENETIC DAMAGE INDUCED BY PESTICIDES USING CYTOGENETIC AND BIOMARKERS ASSAYS IN *Allium* AND *Pisum*

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Currently, due to an increase in the population worldwide, there has been an urge for increasing the productivity of crops and agricultural by-products. This yield increment has been obtained mainly by a massive use of pesticides to control the impacts of noxious insects, pyhtopathogens and weeds in agriculture. Pesticides are a multimillion dollar market corresponding to an estimated value of 25.6 million dollars per year (Cardoso *et al*., 2010) and their application is still the most effective and accepted means for the protection of plants from pest. However, a series of deleterious effects on environment safety and human health have become apparent, where teratogenic, carcinogenic, and mutagenic effects have received special attention (Giacomazzi and Cochet, 2004; Nguyen-Ngoc *et al*., 2009). It is also known that certain pesticides are promutagens which are metabolized to mutagens (Cardoso *et al*., 2010). Because of the undesirable side effects of pesticides, there has been an increase in consumer awareness to avoid the use of these compounds (Zucchi *et al*., 2008) and/or public pressure to enhance their regulation for applications in pre- or post-harvest.

The majority of pesticides have been tested in a wide variety of mutagenicity assays covering gene mutation, chromosomal alteration and DNA damage (Inceer and Beyazoglu, 2000; Soliman and Ghoneam, 2004; Sousa *et al.,* 2009; Cardoso *et al*., 2010; Lamsal *et al*., 2010). Although a number of biomarkers are available to assess transient and permanent genotoxic responses, biomonitoring studies on non target populations exposed to pesticides have essentially focused on cytogenetic end-points, namely chromosomal aberrations (CA), micronuclei (MN) frequency and sister-chromatid exchanges (SCE).

Several investigators had studied the side effect of the pesticides on the heredity material of different plant cells (Soliman and Ghoneam, 2004; Lamsal *et al*., 2010). In particular, *Allium cepa* possess many advantages in the field of environmental mutagenesis for screening of genotoxic agents according to the standard protocol for the plant assays established by the International Program of Chemical Safety (IPCS) and the World Health Organization (Soliman and Ghoneam, 2004). *Pisum sativum* bioassay has been also

Egypt. J. Genet. Cytol., 39: 271-289, July, 2010

shown to be a very good plant bioassay for assessing chromosome damage both in mitosis and meiosis induced by chemicals, radiations and environmental pollutants (Grant and Owens, 2001). The use of plant root tips as a bioassay test system in the genotoxicity of pesticides has shown extremely good correlation with the bacterial and mammalian systems and could exhibits a good predictive value for human beings (Gopalan, 1999; Sadowaska *et al.*, 2001; Soliman and Ghoneam, 2004).

In general, root development is initiated at the apex of the root tip by mitotic divisions in the meristematic regions (about 1 mm) in length above the root cap and the daughter F_1 cells about 1mm are moved upward to lengthen the root structure. A very small portion of the meristematic cells divide transversely to increase the girth of the root tip, whereas the majority divides longitudinally. Based upon this ontogenetic scheme (Ma *et al.*, 1995), the majority of micronuclei should be in F1 except on some rare occasions, when there is a mitotic delay. In most of earlier studies, the Micronuclei (MN) frequencies were probably not scored from F1 cells. Thus MN frequencies obtained in this way would lose their fidelity and the efficiency of the test system would be reduced because the sensitivity of chromosomes to clastogens varies greatly throughout the mitotic cycle (Ma *et al.*, 1995). Among the different cytogenetic assays in plants, the most effective and simplest indicator of cytological damage is micronucleus formation (Ma *et al.*, 1995; Minissi and Lombi, 1997). Furthermore, the micronucleus test can also detect very weak mutagenic effects.

Among the pesticides used, herbicides play a crucial role in agricultural fields to avoid crop competition by weeds (Saladin *et al.*, 2003). On the other hand, chemical insecticides are widely used in Egypt and other countries in the modern agriculture in order to minimize the loss in economic crops due to insect invading (Barakat, 1997).

In view of the mentioned reasons, it was thought of interest, in this work to investigate the cytological and biochemical effects of two different pesticides namely, the herbicide bentazone and the insecticide lannate, using the following approaches: 1) Micronucleus assay in root tips of both *Allium cepa* and *Pisum sativum*, 2) Monitoring of meiotic irregularities in *Pisum* plants and 3) biochemical analysis of the yielded M1 seeds of the treated *Pisum* plants which include assessment of: storage protein banding patterns using SDS-PAGE , protein and nucleic acids content.

MATERIALS AND METHODS

Plant materials

Seeds of *Pisum sativum* L. (Master B) and bulbs of *Allium cepa* (Giza 20) were kindly supplied by the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

Pesticides

Two pesticides, commonly used in the Egyptian agriculture, were tested in the present study: the herbicide bentazone and the insecticide lannate. Both chemicals have been kindly supplied by the General Administration of Pesticides, Ministry of Agricultural of Egypt.

Experimental design

Laboratory assay

Onion bulbs and *Pisum* seeds were germinated as described by Fiskesjo (1988) and Grant and Owens (2001), respectively. When the roots of *A. cepa* L. and *P. sativum* were about 2 cm long, they were exposed to freshly prepared test solutions of different concentrations (500, 1000, 1500 ppm) at room temperature for six hours and maintained in tap water for 44 hours for root germination and recovery after treatment (Ma *et al.*, 1995). Tap water was used as a negative control.

In situ assay

The germinated seeds of *Pisum* were cultivated in pots (30 cm in diameter) containing equal amounts of mixed soil (sand: clay, 1:2 v/v). Ten seeds were sown in each pot and the irrigation was carried out as practiced by adding equal amounts of water to each pot. The pots were divided into five groups; the seeds of the first group were sprayed with tap water to serve as control. The other groups were sprayed twice with concentrations of 500 ppm and 1000 ppm of each pesticides; the spraying were done twice at both seedling and flowering stage before anthesis.

Cytogenetic analysis

Micronucleus assay (MN)

The MN assay was conducted according to Ma *et al.* (1995). After treatments, the root tips were fixed immediately in aceto-alcohol (1:3) and then transferred to 70% alcohol and stored in refrigerator until use. For examination, the root tips were hydrolyzed in 1N HCL at 60°C for 3-8 min and stained in double stain of carbol fuchsin and acetoorcein. Root tips were then squashed in 45% acetic acid. Slides were made permanent and mounted in Canada balsam, examined and photographed.

Meristematic and F1 cell regions were prepared from each root tip separately. The mitotic indices of both the meristematic and F1 cell regions were determined (no. of dividing cells/total no. of examined cells X 100). The micronuclei were scored per 1000 interphase cells from 5-7 separate slides and expressed in terms MN/1000 cells. A treated/ control (T/C) ratio was used to compare the efficiency of the MN scoring from the meristematic and F1 cell regions of the root tips and also to compare the efficiency of MN scoring from the two plant tests (Ma *et al.*, 1995).

Meiotic analysis

Six flowering small buds from each treatment were collected 24 hour after the

last spray. Flower buds were fixed in (6:3:1) ethanol: glacial acetic acid: chloroform (Carnoy's fluid) and then preserved in 70% ethyl alcohol and stored at 4°C until cytological analysis. Staining and smearing were made according to Snow (1963). The various types of cells with normal and abnormal chromosomal behavior at both the 1st and 2nd meiotic divisions and tetrad stage were observed and counted.

Biochemical analysis

M1 seeds were used for biochemical analysis which included: storage protein banding patterns as well as measurement of both protein and nucleic acids content.

Estimation of total proteins and nucleic acids

Protein content was extracted from dry M1 seeds according to Bradford (1976). The protein content in the seed extract was determined by spectrophotometer. The quantity of protein in the M1 seeds of *Pisum sativum* samples was calculated from the standard curve using Bovine Serum Albumin solution as standard protein.

A method based on that of Shibho *et al*. (1967) was performed for estimation of both types of nucleic acids. RNA content was determined according to Ashwell (1957). The method of Burton (1968) was used to determine DNA content.

SDS-PAGE of seed protein

Total extracted proteins of M1 seeds of *Pisum* plants were separated by denatured polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli (1970). Gels were stained with Comassie blue. The banding profile in the gel was photographed and analyzed using Gel documentation system.

Statistical analysis

The data were statistically analyzed using a SPSS program (Nie *et al*., 1975). Linear regression dose-response curves were established and the coefficients of correlation and slope of the dose–response curve were calculated to mark the efficiency of the system as a clastogenicity bioassay. All experiments were repeated at least three times.

RESULTS AND DISCUSSION

Cytogenetic analysis

The obtained data elucidated that, the two tested pesticides induced different mitotic changes on root tips of *P. sativum* and *A. cepa* appeared as a low mitotic index and a high frequency of induced micronuclei, suggesting the genotoxicity of the studied pesticides. Screening of pesticides in different bioassays systems revealed that some pesticides may have known or unknown mutagenic or carcinogenic effects (Tartar *et al*., 2006; Lamsal *et al*., 2010). The use of plants bioassays for studying the chromosome aberrations induced by different classes of pesticides

is one of the oldest, simplest, most reliable and inexpensive methods available (Lamsal *et al.*, 2010; Cardoso *et al.*, 2010).

Micronucleus assay

Among the different cytogenetic assays in plants, the most effective and simplest indicator of cytological damage is micronucleus formation (Ma *et al.*, 1995). Furthermore, the micronucleus test can also detect very weak mutagenic effects (Minissi and Lombi, 1997). In this study, all the concentrations of lannate and bentazone were capable to induce a significant increase in percentage of total MN in interphase cells of *P. sativum* and *Allium cepa* root tips relative to control (Plate 1 and Fig. 1). This increment of MN frequencies in F1 and meristem cells (M) of *Pisum* and *Allium* was dose-dependent. On the other hand, *Allium* cells exhibited higher percentage of MN in both F1 (20-161%) and M (6-41%) cells compared to *Pisum* (15-117% in F1; 3-26% in M cells).

Since the MN are revealed in the subsequent generation in the interphase or prophase cells (F1 cells). Ma *et al.* (1995) suggested that efficient scoring should not be done in the meristematic mitotic cells and therefore a long recovery time covering two rounds of mitosis after treatment is required. This is designed to capture the maximum chromosome damage which was inflicted in the interphases (G1, S and G2) of the meristematic cells and later becomes nuclei in the F1 cell population (Ma and Xu, 1986). Some researchers scored MN from interphase cells and others scored MN together with chromosome abnormalities. The inconsistent use of one method over the others has reduced the efficiency of the test system. In order to increase the fidelity of the test system which scores MN in the F1 cells, data collection should be centered at the sensitive peak stage for the induction of chromosome breaks. According to Ma *et al.* (1995), the sensitive peak was represented by 44-h recovery period which gives the highest MN frequency. Based on the 24-h mitotic cycle in the roots, the 44-h recovery time captures the MN which were induced around the G1 or S stage of the parental meristematic cells and appeared in the interphase or prophase in the daughter cells.

Judging from the slope values of the dose-response curves (Fig. 2), it is clear that greater efficiency can be attained when the MN frequencies are scored from the F1 cell region rather than from the general meristematic region. F1 region showed both a higher slope value (0.031-0.1042) and correlation coefficient (R^2 = 0.8491-0.9976) compared to meristematic region (slope values = 0.0068-0.0256 and R^2 = 0.7506-0.9537). There is a greater degree of synchrony of interphase cells in the F1 region than in the meristematic region, which facilitates the MN scoring. Scoring MN from F1 cells has the advantage of recording both chromosome breaks which are generated in the early G1 and the late interphase (G2) of the parental cells when conventional 44-48 h recovery time is used. On the other hand, the treated/control (T/C) value

showed a higher fold increase in F1 cells than in M cells in both *Pisum* and *Allium* root tips (Table 1), confirming the high efficiency of using F1 cell populations for MN scoring. These findings were in accordance with that of Ma *et al.* (1995) where scoring MN was more efficient in F1 cells in both *Vicia* and *Allium* root tips.

Mitotic activity

Concerning the effect of lannate and bentazone treatments on cell division, the result showed that increase in the pesticide concentration decreases the mitotic indices significantly in both *P. sativum* and *A. cepa* root meristems compared to the control values (Fig. 1). It is clear that the reduction in mitotic activity in the root tips of *A. cepa* was more pronounced than in *P. sativum*. Such an inverse relation between the mitotic index and the dosage of treatment had been reported earlier by various workers studying different pesticides on various plant materials (El Nahas, 2000; Lamsal *et al*., 2010). The changes of mitotic activity in plant have been attributed to many factors (Kim and Bendixen, 1987; Lamsal *et al*., 2010): 1) the inhibition of certain types of nuclear proteins essential in the mitotic cycle, 2) the inhibition of DNA synthesis and 3) the reduction of various necessary metabolites which are important for transition from G1 to S and from G2 to mitosis.

Sensitivity test

It is evident from the slope values of the dose- response curves (Fig. 2), that *Allium* root MN was a more efficient test than the *Pisum* one. The relatively high efficiency of the *Allium* test is substantiated by the T/C values at all concentrations of both pesticides (Table 1). The T/C values showed higher fold increase in the percentage of micronuclei in F1 (4-32.2) and M (2-13.7) of *Allium* cells compared to T/C values of the MN frequencies in F1 (1.9-14.6) and M (1.5-13) of *Pisum* cells. Thus it was concluded that *P. sativum* was not as sensitive as *A. cepa* in detecting clastogenicity of the tested pesticides. The higher sensitivity of *Allium* could be attributed to its large size of chromosomes and this may led to greater susceptibility of *Allium* chromosomes to breakage (Grant and Owens, 2001).

Meiotic division

Statistical analysis of the results (Table 2) showed that all of the conducted treatments with both pesticides caused induction of significant percentage of abnormal pollen mother cells (PMC's)/plant in the first and second meiotic division (Plate 2). Bentazone treatments caused a higher percentage of abnormal PMC's as compared with lannate. Furthermore, the frequencies of total meiotic abnormalities were higher in the first division than in the second one in all treatments. These results indicate the potentiality of the investigated pesticides to induce meiotic irregularities. Similar results have been recorded by other authors (El-Nahas, 2000; Soliman and Ghoneam, 2004).

Stickiness, laggards, bridges, micronucleus and disturbed chromosomes were the main types of anomalies ob-

served (Fig. 3 & Plate 2). The frequencies of these abnormalities depend on both the applied concentrations and the pesticide used. The most prominent anomaly observed, particularly in metaphase I was the sticky nature of chromosomes (Plate 2A). It is evident from Fig. (3) that the percentage of cells with sticky chromosomes reached a maximum frequency of 90% of the abnormal PMC's after treatment of lannate at 1000 ppm concentration compared with the bentazone value (75.20%). Stickiness has been suggested as a type of physical adhesion involving mainly the proteinacious matrix of chromatin material (Lamsal *et al.*, 2010). On the other hand, laggards appeared in a considerable frequency in both the first and second meiotic divisions as one of the bivalents failed to reach the equatorial plan and remains free in the cytoplasm. Induction of laggards at metaphase I may be extended in all the subsequent meiotic stages, therefore laggards appeared at anaphase I and II (Plate 2 B & E). The highest percentage of laggards (20.20%) was observed at 500 ppm lannate. The induction of laggards could be attributed to the failure of the spindle apparatus to organize and function in a normal way (Patil and Bahat, 1992). Other type of abnormalities was the formation of anaphase bridges which appeared in a low significant percentage (Plate 2D). The percentage of bridges reached its maximum value of 4.50% at 500 ppm lannate (Fig. 3). The formation of bridges could be attributed to the general stickiness of chromosomes (Soliman and Ghoneam, 2004; Lamsal *et al.*, 2010). A less common phenomenon was the appearance of some PMC's with micronuclei (Plate 2C). The highest proportion of such abnormality recorded was 8.30% at bentazone 1000 ppm (Fig. 3). Fewer proportions of PMC's with disturbed poles were recorded at the 2nd meiotic division, in which the equatorial planes at metaphase II and anaphase II (Plate 2F) disoriented in an abnormal fashion. The total frequency of disturbed cells reached a maximum value of 11.4% after spraying with 1000 ppm bentazone (Fig. 3). This abnormality is generally regarded to be the result of disturbance of spindle apparatus (Yuzbasioglu *et al.*, 2003).

Biochemical analysis

Changes in DNA and RNA contents

The present results of DNA and RNA content are confirmed by data obtained by other studies on different pesticides (Badr and Ibrahim, 1987; Topkatas and Rencuzogullari, 1996). Nucleic acid biosynthesis has been reported as a potential target sites affected by herbicides which in turn led to inhibition of protein synthesis (Soliman and Ghoneam, 2004). The mutagenic effect of the investigated pesticides on the genetic material of *P. sativum* M1 seeds is confirmed by its effect on the nucleic acids. Statistical analysis of the results revealed that all the conducted treatments caused significant reduction in the DNA and RNA contents in pea yield (Fig. 4). DNA concentrations ranged from 85.91-110.73 µg/100g dry wt. compared with the control (120.72 µg/100g dry wt.), while the amounts of RNA ranged from 175.41 to 205.63

µg/100g dry wt as referred to control (219.40 µg/100g dry wt). In general the magnitude of decrease in the amounts of DNA and RNA was most pronounced at bentazone 1000 ppm, (85.91 µg/100g dry wt. and 175.41 µg/100g dry wt.) for DNA and RNA, respectively (Fig. 4). The inhibition of DNA and RNA synthesis by herbicides could be attributed to the inhibition of DNA replication, or due to the reduction of oxidative phosphorylation resulting in lower ATP level in the cell (Lamsal *et al.*, 2010). On the other hand, herbicides that reduce ATP have been postulated to be strong inhibitors of RNA synthesis (Ashton and Crafts, 1981).

Changes in protein contents

Soluble protein content in organisms is known to respond to a wide variety of stressors (Singh and Tewari, 2003). It was proposed that certain pesticides caused a significant reduction of protein level in plants (Mosleh *et al.*, 2003; Singh and Tewari, 2003). In this experiment, changes in soluble protein content of *Pisum* seeds (M1), exhibited an inverse relationship with bentazone treatments. On the other hand, other investigators have recorded an increase in protein content in response to metal stress (Liu *et al.*, 2007). Here, a positive correlation between protein content and lannate concentrations was remarkable (Fig. 5). Values of protein contents in both situations were statistically different from the control indicating its potential as a biomarker of reversible and irreversible changes in metabolism. The maximum value (22487.71 µg/100g. dry wt.) was observed after treatment with lannate 1000 ppm while the minimum value (19779.25 µg/100g dry wt.) was observed after treatment with bentazone 1000 ppm (Fig. 5).

Changes in protein banding patterns

SDS-PAGE analysis was successfully used by some authors to establish biochemical genetic fingerprints of many plants (Badr, 1995; Ghareeb, 1998). The protein profile of M1 seeds of *Pisum* plants (treated and untreated) using SDS-PAGE gel is illustrated in Plate (3). Their molecular weights (MW), relative front, number of bands and average optical density (OD) are given in Table (3). The protein patterns showed qualitative and quantitative variations among the investigated samples. The variations included: the appearance of new bands, changes in band intensity, and changes in band relative mobility. Similar results were obtained by other authors (El-Nahas, 2000; Babaoglu *et al.*, 2004).

The number of bands in the studied samples ranges between 8 and 10 bands in treated samples with molecular weights ranging between 273.652 and 88.254 KDa (Table 3). The most obvious changes in SDS-PAGE profile of M1 seeds of the treated plants were; the appearance of new band at lannate 500 ppm (94.606 KDa), three bands at lannate 1000 ppm (94.675, 90.768 and 88.254 KDa) and other new band at both bentazone 500 and 1000 ppm (95.460 KDa). The appearance of new bands may be explained on the basis of mutational events at the regulatory system

of an unexpressed gene(s) that activate it (El-Nahas, 2000). Also, pesticides used in this investigation caused changes in band intensity which could be explained on the basis of induction of gene mutation at the regulatory system which modulate or increase transcription rate of a particular structural gene. This lead to the production of faint or over expressed protein bands (Barakat and Hassan, 1997). Moreover, the recorded changes in band intensity could be also attributed to the cytological abnormalities induced by pesticides (Soliman and Ghoneam, 2004). The presence of laggards and bridges support this conclusion.

Changes in bands relative mobilities could be also interpreted on basis of the occurrence of point mutation in the concerned structural genes that create stop codon prior or post the original. They gave rise to the production of shorter or longer polypeptide chains (Ghareeb, 1998; El-Nahas, 2000).

It is worthy to mention that gene mutation and changes in the gene expression have to be considered as the reasonable interpretation for the observed banding pattern changes. The correlation between the alteration in the protein banding patterns and mutational events has been demonstrated by other authors (Prasad and Zha, 1992; El-Nahas, 2000; Soliman and Ghoneam, 2004). Thus, the two investigated pesticides in the present work might be considered as strong mutagens and the observed changes in the banding patterns are heritable changes, as the treated parents were capable to transfer these mutational events to the next generation.

As a general conclusion, the two pesticides (bentazone and lannate) caused harmful effects at both cytogenetic and biochemical levels on the plants. However, the effect of bentazone was more pronounced in this regard than lannate. Therefore, it is suggested to test the mutagenic potential of pesticides on a more intensive and extensive basis, especially on non target systems before it is recommended for wider use in agricultural field. This study emphasizes that the programmes of pesticides application should also take into account the concentration of the pesticides and the nature of plant.

ACKNOWLEDGMENTS

The authors are thankful to the authorities of Mansoura University for providing facilities and cooperation in carrying out this work, which is a part of major research of granted scholarship sponsored by the University.

SUMMARY

The present investigation has been conducted to detect the possible genetic damage caused by two pesticides, namely, the lannate insecticide and the bentazone herbicide, on non-target organisms. Cytogenetic analyses were performed in the mitotic cell division of *Allium cepa* and *Pisum sativum* and meiotic pollen mother cells of *Pisum sativum.* Biochemical analyses were also performed in M1 seeds

of *Pisum sativum* to assess the possible changes in nucleic acids and proteins. Laboratory assay included treatment of *Allium* and *Pisum* root tips with three concentrations (500, 1000 and 1500 ppm) of each pesticides for six hours followed by 44 h recovery period, for conducting Micronucleus (MN) assay. *In situ* assay included spraying *Pisum* plants at seedling and flowering stage with two concentrations (500 and 1000 ppm) of pesticides. Cytogenetic analysis proved the efficiency of conducting Micronucleus assay in F1 cells relative to the meristematic region of root tips. Results of MN assay also exhibited *Allium* a more sensitive test compared to *Pisum.* Spraying the flower buds of *Pisum* plants with the applied concentrations of the two pesticides resulted in a significant increase of total abnormal PMC's. The types of meiotic abnormalities induced were: stickiness, lagging, bridges, micronucleus and disturbed chromosomes. Results of biomarkers assay revealed a pronounced mutagenic effect on the hereditary material of *Pisum* M1 seeds confirmed by reduction in the DNA and RNA contents and alteration in protein synthesis. In addition, the protein banding patterns of M1 seeds exhibited both qualitative and quantitative changes. Overall, it can be concluded that the two pesticides have potential mutagenic effects on genetic material at different levels. So, it will be necessary to test the mutagenic potential of bentazone and lannate on a more intensive basis especially on non-target systems before it is recommended for wider use in agricultural field.

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Table (1): T/C values at different concentrations of lannate and bentazone estimated from micronuclei frequency in F1 and M cells of both *Allium* and *Pisum.*

Concentration (ppm)		T/C (X)			
		Allium		*Pisum*	
		F1	M	F1	M
0 (control)		1.0	1.0	1.0	1.0
Lannate	500	4.0	2.0	1.9	1.5
	1000	7.4	6.3	2.9	2.0
	1500	13.0	9.3	7.1	6.5
Bentazone	500	6.4	3.3	3.9	3.5
	1000	17.0	8.0	9.3	7.5
	1500	32.2	13.7	14.6	13.0

Table (2): Percentages of abnormal pollen mother cells (PMC's) in the different meiotic stages in *Pisum* plants sprayed with different concentrations of lannate and bentazone.

Concentration (ppm)		Total No. of PMC's	Total No. of abn. PMC's	% abnormalities							
				Division I				Division II			
				Meta.	Ana.	Telo.	Total	Meta.	Ana.	Telo.	Total
0 (control)		1500	31	4.00	4.60	0.52	2.58	4.57	1.45	0	1.44
Lannate	500		375*	37.11*	39.58*	10.40*	26.04*	48.34*	39.11*	0.33*	24.20*
	1000		494*	75.66*	61.08*	29.94*	51.41*	76.30*	57.71*	0.68*	37.83*
Bentazone	500		414*	46.20*	56.40*	19.86*	43.05*	65.22*	54.60*	0	30.85*
	1000		792*	65.42*	62.87*	21.41*	61.80*	66.16*	55.52*	0	49.91*
LSD 5%				4.46	3.13	1.57	1.79	0.80	2.01	0.08	1.96

* Significant at 0.05 LSD.

Table (3): Data of SDS-PAGE analysis of M1 seeds of *Pisum sativum* plants treated with different concentrations of lannate and bentazone (a: Mwt. KDa, b) Relative front; c) Average of O.D. of bands).

a) Molecular weight (MW) KDa

Band no.	Concentration (ppm)				
	0 (control)	Lannate		Bentazone	
		500	1000	500	1000
1	273.652	273.652	273.652	273.652	273.652
2	243.912	243.912	243.912	243.912	243.912
3	178.230	178.230	178.230	178.230	178.230
4	141.038	141.038	141.038	141.038	141.038
5	114.592	114.592	114.592	114.592	114.592
6	108.722	108.722	108.722	108.722	108.722
7	100.177	100.177	100.177	100.177	100.177
8	-	94.606	94.606	59.091	95.460
9	-	-	90.768	-	-
10	-	-	88.254	-	-

b) Relative Front (RF)

Band no.	Concentration (ppm)				
	0(control)	Lannate		Bentazone	
		500	1000	500	1000
1	0.053	0.044	0.044	0.041	0.047
2	0.085	0.082	0.075	0.073	0.073
3	0.166	0.164	0.154	0.146	0.149
4	0.219	0.220	0.211	0.206	0.203
5	0.285	0.292	0.299	0.272	0.269
6	0.357	0.358	0.362	0.345	0.342
7	0.473	0.475	0.481	0.465	0.456
8	-	0.550	0.557	0.538	0.522
9	-	-	0.613	-	-
10	-	-	0.651	-	-

c) Average of Optical Denisty (O.D.)

Band no.	Concentration (ppm)				
	0(control)	Lannate		Bentazone	
		500	1000	500	1000
1	0.306	0.449	0.492	0.453	0.286
2	0.307	0.430	0.503	0.445	0.278
3	0.343	0.435	0.505	0.465	0.306
4	0.306	0.397	0.490	0.438	0.305
5	0.273	0.413	0.479	0.412	0.268
6	0.319	0.415	0.440	0.412	0.302
7	0.205	0.331	0.351	0.334	0.209
8	-	0.283	0.319	0. 303	0.219
9	-	-	0.304	-	-
10	-	-	0.246	-	-

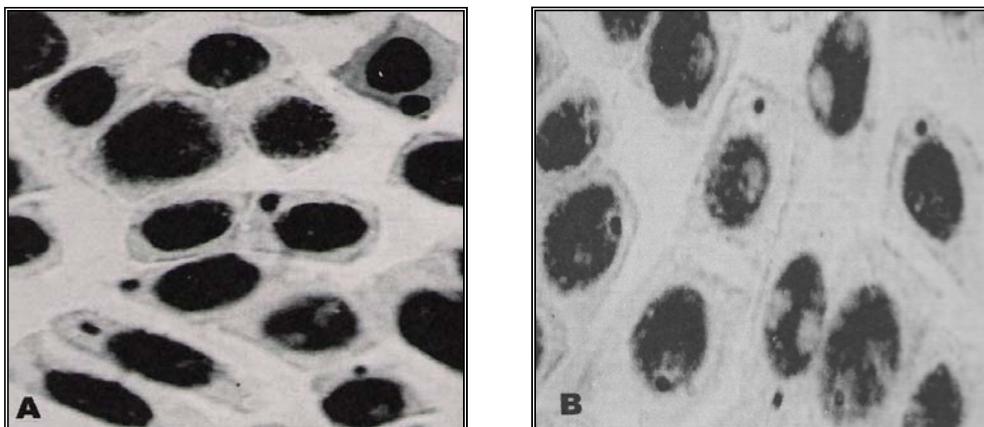


Plate (1): Micronuclei in interphase cells of (A) *Allium cepa* and (B) *Pisum sativum* root meristems after treatments of lannate and bentazone.

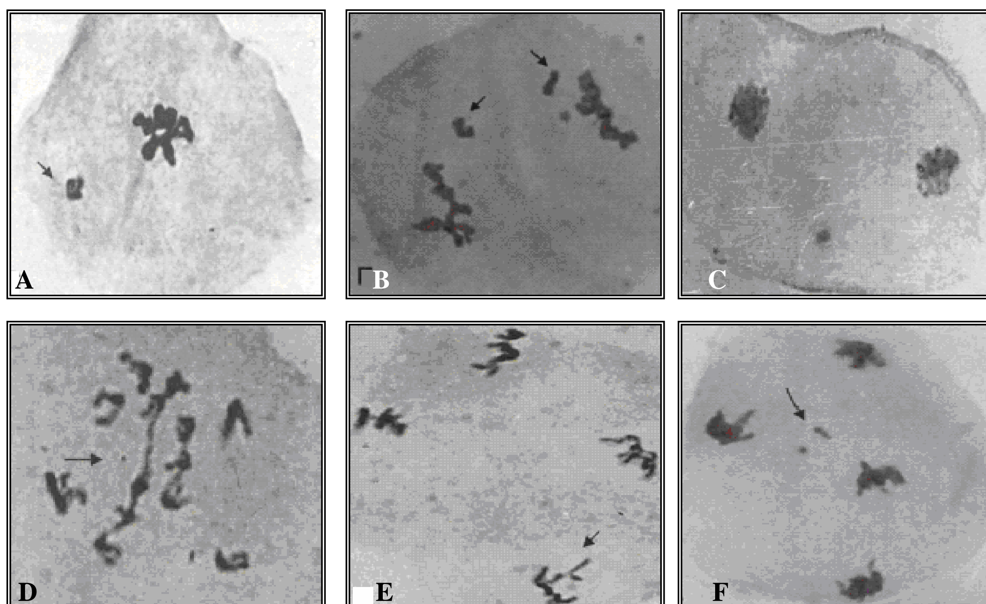
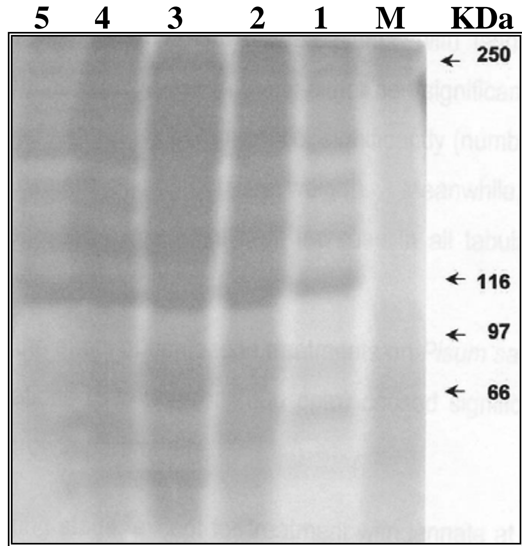


Plate (2): Different kinds of abnormalities in pollen mother cells of *Pisum sativum* A: Sticky metaphase I; B: Laggards in anaphase I; C: Micronucleus in telophase I; D: Bridges in anaphase II; E: Laggards in anaphase II; F: Disturbed anaphase II (misorientation and Laggards).

Plate (3): Electrophotograph produced by denatured SDS-PAGE analysis of general protein patterns of M1 seeds of *Pisum sativum* plants sprayed with different concentrations of lannate and bentazone Lane M: protein molecular wt. marker. Lane1: Control: lane 2and 3: Lannate 500, 1000 ppm, respectively; Lane 4 and 5: bentazone 500 and 1000 ppm, respectively.



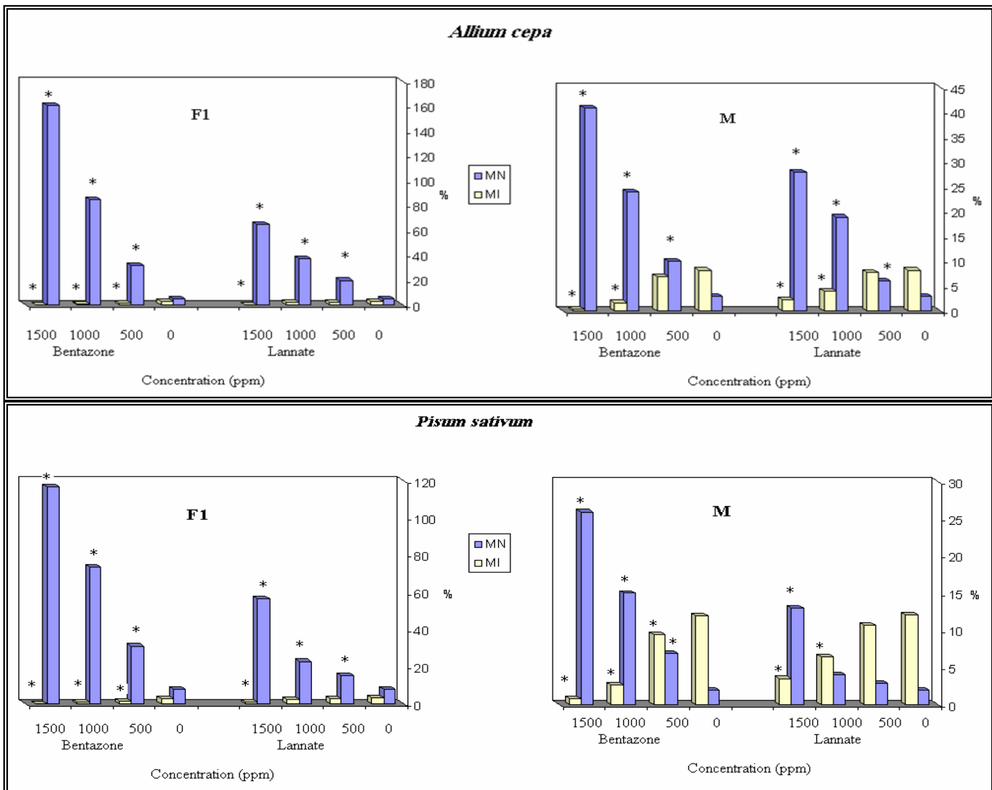


Fig. (1): Micronuclei frequencies (MN), mitotic index(MI) scored in F1 and meristem (M) cells of treated meristems of *Allium* and *Pisum* at different concentrations of bentazone and lannate. * = significant at 0.05 LSD.

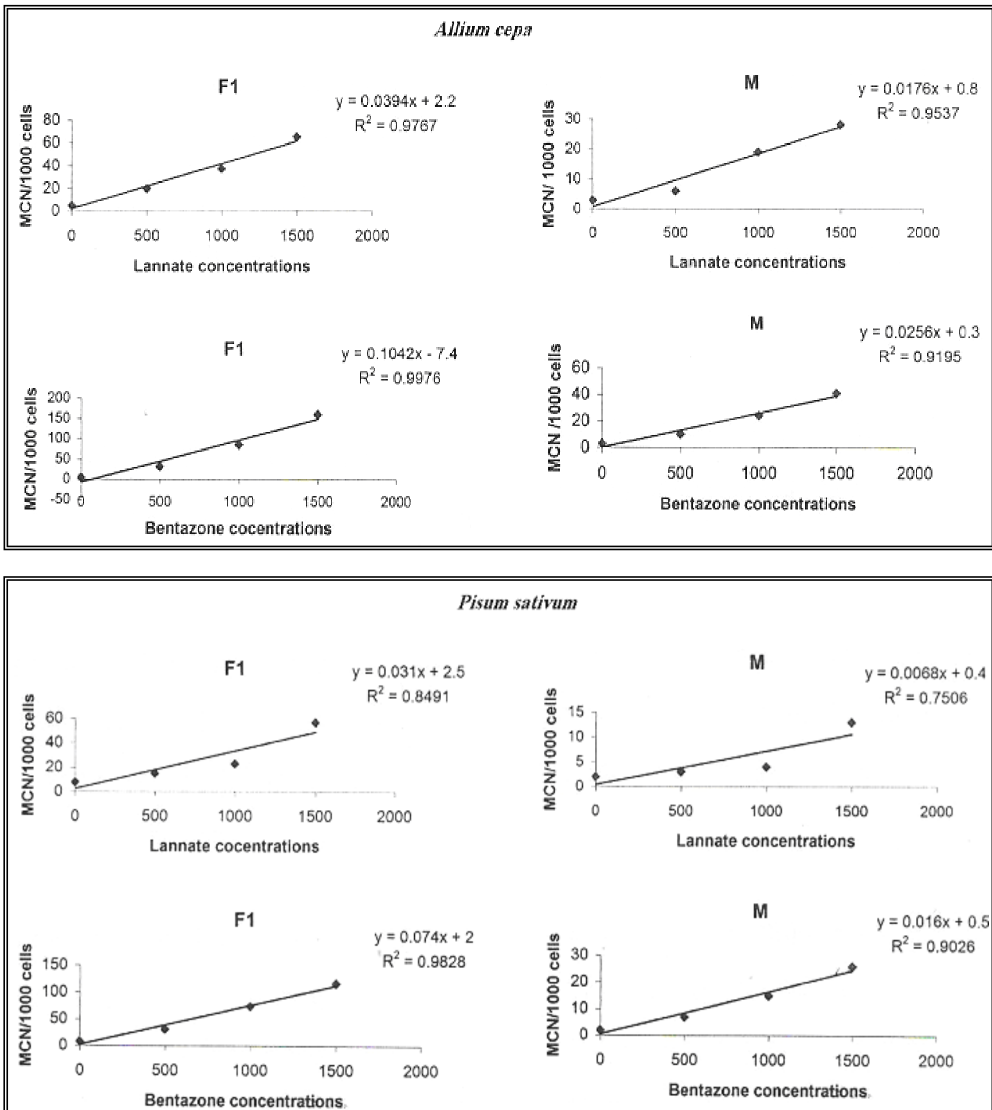


Fig. (2): Linear dose-response curves for micronucleus frequency in F1 and M cells of the treated meristem of *Allium* and *Pisum*.

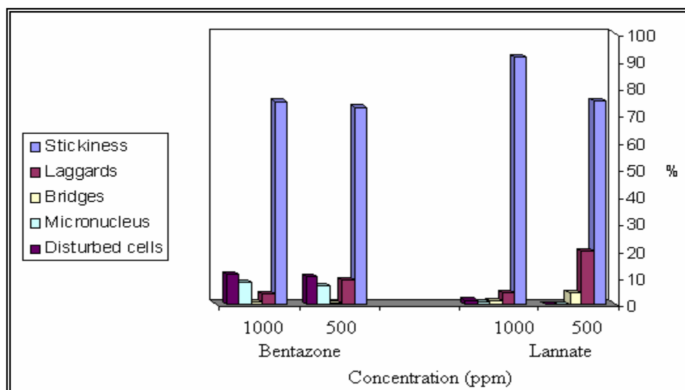


Fig. (3): Percentage of the different types of abnormalities in *P. sativum* PMC's sprayed with different concentrations of lannate and bentazone.

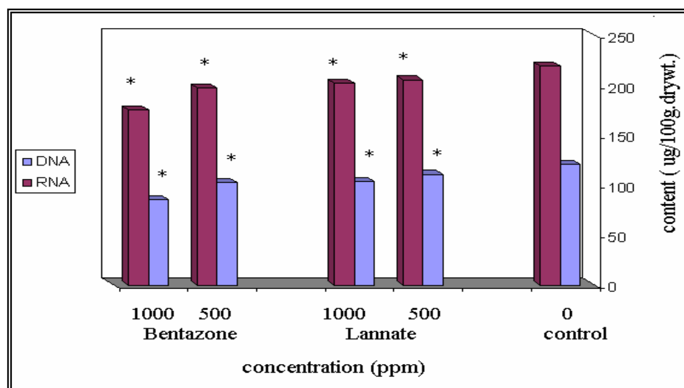


Fig. (4): Changes in nucleic acids content (DNA & RNA) in *Pisum* M1 seeds in response to treatments of bentazone and lannate. * = significant at 0.05 LSD.

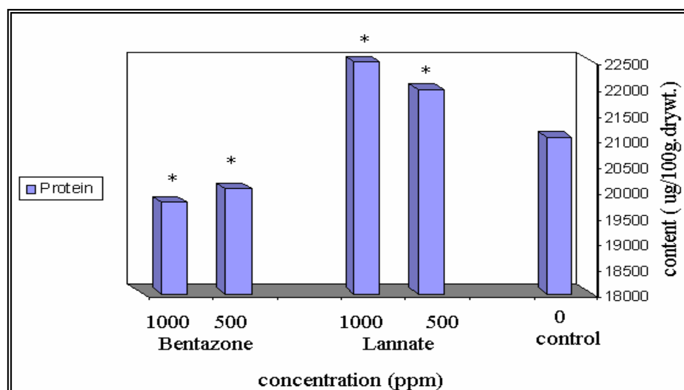


Fig. (5): Changes in protein content in *Pisum* M1 seeds in response to treatments of bentazone and lannate. * = significant at 0.05 LSD.