



### Full Length Article

# The Effect of Three Agricultural Chemicals on Mitotic Division and Total Seed Protein Banding Profiles of Alfalfa (*Vicia faba*)

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## ABSTRACT

Treatments of alfalfa (*Vicia faba* L.) seeds with gibberellic acid (GA<sub>3</sub>), fertilizer, potassium oxide (K<sub>2</sub>O) and the auxin inhibitor N-meta-tolyl-phthalamic acid (Tomaset) showed a decrease in mitotic index and increased the percentage of the chromosomal abnormalities. The harmful effect was high with the use of tomaset, moderate with K<sub>2</sub>O and low in plants treated with GA<sub>3</sub>. SDS-PAGE studies revealed qualitative and quantitative variation in seed protein among the treatments. The number of polypeptide bands varied from 25 bands in control plants to 15 bands in plants treated with 2X tomaset. A different specific polypeptide band was observed as a newly synthesized band with each chemical, a band with (MW) 22.04 kDa and very faint small band with MW 14.5 kDa was induced with the use of K<sub>2</sub>O, 39.7 kDa band induced in seeds treated with tomaset, while the band 48.9 kDa was observed in control and seeds treated with the higher concentration of GA<sub>3</sub> only. The results showed that tomaset was the most harmful chemical used.

**Key Words:** Seed treatment; SDS-PAGE; Chromosomal abnormalities; Seed protein

## INTRODUCTION

The use of plant growth regulators (PGRs) and other agricultural chemicals has become a routine procedure for growers in all regions of the world. Most studies on the effect of these compounds on plants are concerned only with the desirable effects such as their influence in the vegetative growth, flower formation, fruit quality. However, studies on the harmful effect of these chemicals are often ignored.

Gibberellic acid has been used to stimulate cell division and elongation (Francis & Sorrell, 2004), break seed dormancy and increase the number of leaves, flowers, fruits and seeds of many plants (Harb, 1992; Abd-El Hameid *et al.*, 1999; Sarkar, *et al.*, 2002; Abdul Hye *et al.*, 2002; Hossain *et al.*, 2006). Shukry and El-Bassiouny (2002) reported on the importance of GA<sub>3</sub> in overcoming the harmful effect of seawater salinity. It is also proved useful in counteracting the adverse effect of heavy metals (Mansour & Kamel, 2005). On the other hand the effect of GA<sub>3</sub> in inducing abnormal features in barley ears was detected by Kirby (1971). The use of high concentrations of GA<sub>3</sub> increased the proportion of either abscised or inactive buds, resulting in fewer indeterminate inflorescences and yield reduction (Salazar-Garcia & Lovatt, 1998 & 99). DeMason and Chawla (2006) reported that auxin and GA inhibitors produce common abnormalities during pea leaf development. GA exerts its physiological effect such as altered auxin status of tissue it acts at the gene level to cause

depression of specific gene (<http://www.ikisan.com/links/apregulators.shtml>).

Potassium Oxide (K<sub>2</sub>O) is a liquid fertilizer used to improve building of protein, photosynthesis and fruit quality. It is absorbed by plants in large amount than any other mineral elements except nitrogen. It has a role in influencing the uptake of certain other mineral elements and influencing the action of enzymes (Foy & Barber, 1958). Tomaset (N-meta-tolyl-phthalamic acid) is an auxin inhibitor commercialized in the early 1990s as a type of PGRs for producing parthenocarpic fruits or fruits with reduced seed number <http://www.freepatentsonline.com>. The use of Tomaset induced significant increase in flower and fruit numbers per inflorescence but the severe deformative action produced by this compound and its side effects reduced the usefulness of this chemical (Morgan, 1986).

The aim of the present study was to investigate the effect of the growth hormone; gibberellic acid (GA<sub>3</sub>), fertilizer; (Potassium Oxide; K<sub>2</sub>O) and the auxin inhibitor N-meta-tolyl-phthalamic acid known as Tomaset on cell division, chromosomes and total seed protein electrophoretic bands of alfalfa (*Vicia faba* L.).

## MATERIALS AND METHODS

Seeds of alfalfa (*Vicia faba* L.Cv. Bonyards exhibition) were soaked for 12 h. in water and then transferred to Petri-dishes containing moist filter papers,

after two to three days of growth the roots were treated with three different concentrations of GA<sub>3</sub>, K<sub>2</sub>O and Tomaset. Three concentrations of these compounds corresponding to (0.5X, 1X & 2X) were applied for 12, 24 and 36 h. The X dose for GA<sub>3</sub> was 125 ppm and for the Tomaset and potassium oxide was 1000 ppm and is the recommended concentration for use in the field. Control roots were simultaneously treated with distilled water.

**Cytological preparations.** After all treatments root tips were detached, washed several times in water and fixed in 3:1 (v:v) ethanol: glacial acetic acid overnight and stored in 70% ethanol in the refrigerator until use for cytological preparations. Permanent preparations for treated and control root meristematic cells were made using the Feulgen squash method as follows: Roots were removed from the fixative, washed several times with tap water, blotted on filter paper, hydrolyzed in 1 N HCl for 8 min in water bath at 60°C then stained for 2 h in leucobasic fuchsin. Individual roots were then washed and squashed in a drop of 45% glacial acetic acid. Preparations were then dehydrated in ethanol and mounted in Euparal. At least eight slides were prepared for each treatment and control. Mitotic index was calculated by counting the number of dividing and non-dividing cells in at least six slides. In addition, at least 400 dividing cells were examined and the number and types of chromosomal abnormalities in the mitotic phases were recorded.

**SDS-PAGE seed protein.** Total seed protein was analyzed for seeds harvested from plants treated with the three growth regulator chemicals for 24 h. Seeds were crushed using coarse powder. A 0.5 g of which was mixed with equal weight of pure, clean, sterile fine sand and powdered using a mortar and pestle. The samples were homogenized with 5 mL of tris-glycine buffer containing 2% NaCl at pH=8.2 for 2 h at 20°C, by gentle motion and centrifuged at 20,000 × g for 20 min. The supernatant (protein extract) was electrophoresed in mini-slab gel in a tris-borate buffer (pH=8) at 4°C using a low molecular weight protein mixture as a marker in each run. Gels were stained in Coomassie blue R-250 for 30 min., de-stained and photographed, while gel was wet and the tracks of protein bands on the gel electrophotographs were scanned using a densitometer (Jel-Pro-Analyzer ver. 3.3, Media Cybernetic, 93-97) to determine the intensity of each band.

## RESULTS

Cytological studies showed that the use of plant growth regulator GA<sub>3</sub> stimulated the mitotic activity in the roots of alfalfa, since the MI increased with the increase in the concentration as well as the treatment duration. The MI value increased from 15.2 with the use of concentration (0.5 X) for 12 h. to 21 with the use of concentration (2 X) for 36 h (Table I). However, GA<sub>3</sub> treatments induced insignificant amount of mitotic abnormalities compared to control roots.

On the other hand, the use of K<sub>2</sub>O showed little increase in MI with lower duration but MI was decreased

with the increase in the duration. MI of the root tips treated with K<sub>2</sub>O for 12 h. increased from 14.4% with the concentration 0.5X to 15.3% in root tips treated with concentration 2X concentration (Table I). On the other hand the MI decreased to 12.9% in 2X concentration used for 36 h. The data further showed increase of the total percentage of abnormalities with the use of K<sub>2</sub>O than with the use of GA<sub>3</sub> and the control. It ranged from 14.2% with the lowest concentration (0.5 X) for duration of 12 h. to 20.3% with the treatment using the 2X for 36 h.

The use of Tomaset produced the most significant decrease in MI with the increase of the concentration. In roots exposed to this chemical MI was reduced to 6.3. The percentage of the total abnormalities in roots exposed to this compound are severely increased with the use of tomaset compared with the other two chemicals used (GA<sub>3</sub> & K<sub>2</sub>O) and the control. The percentage of total abnormal cells in the root tips treated with different concentration differed from 25.1% with the use of the concentration 0.5X for 12 h. to 64.3% with 2X for 36 h.

The most common types of chromosomal abnormalities induced by the use of tomaset were chromosomal stickiness, anaphase bridge, chromosome breakage, chromosome fragment at anaphase, disturbed anaphase, un-equal divided nuclei, star metaphase, star anaphase and interphase nuclei vacuolation (Fig. 1). The data also showed a significant increase in the percentage of prophase cells with the use of Tomaset from 25.6% cells in control to 50.2% with using the diluted concentration (0.5 X) for 12 h. and increased with the increase of concentration and duration to 83.1% with concentration 2X for 36 h. Chromosomal stickiness, anaphase bridge and C-metaphase were the most abnormal phases recorded with the use of GA<sub>3</sub>. The most common abnormalities found with the use of K<sub>2</sub>O were chromosome stickiness, anaphase bridge, nuclear budding, anaphase with chromatid fragmentation (Fig. 1).

Protein patterns (Fig. 2) and their relative concentration (Table II) with the use of three concentrations of these chemicals for 24 h indicated the occurrence of 32 polypeptide bands. Out of these bands, 25 were found in the control plants. In the plants treated with the use of 0.5X concentration of Tomaset the number of protein bands decreased to 21. The use of K<sub>2</sub>O decreased the number of protein banding to 20 bands with the high and moderate concentrations (1X & 2X) and to 22 bands with the lowest concentration (0.5 X). On the other hand the number of protein bands with the use of GA<sub>3</sub> decreased to 21 with the highest concentration and 24 bands with moderate and low concentrations. It was also evident that the polypeptide bands with the molecular weight (Mw.) 101.99, 48.93 and 44.22 kDa disappeared with the use of the three concentrations of Tomaset as compared with the control. On the other hand, a high intensity protein band with 39.7 kDa was synthesized in the plants treated with the three concentrations of Tomaset, while two bands with molecular mass 142.14 and 95.10 kDa synthesized only with the use of

**Table I. Effects of different treatments of gibberellic acid, potassium oxide and Tomaset on mitotic index, frequency of mitotic phases and percentage of abnormalities in mitotic division of alfalfa root tips**

Agent	duration	conc.	Total cells divided	Total abnormal cells	Abnormal cells (%)	Mitotic index	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Ana- & Telo-phase No.	Ana- & Telo-phase %
<b>Control</b>		<b>H<sub>2</sub>O</b>	<b>620</b>	<b>26</b>	<b>4.2</b>	<b>13.8</b>	<b>159</b>	<b>25.6</b>	<b>165</b>	<b>26.6</b>	<b>296</b>	<b>47.7</b>
<b>GA<sub>3</sub></b>												
	12h	0.5X	685	32	4.7	15.2	174	26.7	187	28.8	289	44.5
		X	714	35	4.9	15.9	159	24.1	199	30.2	302	45.8
		2X	785	43	5.5	17.4	176	26.3	187	27.9	307	45.8
	24h	0.5X	820	41	5.0	18.2	182	26	198	28.3	320	45.7
		X	860	45	5.2	19.1	175	24.6	200	28.2	335	47.2
		2X	882	50	5.7	19.6	169	23.3	194	26.8	362	49.9
	36h		885	42	4.7	19.7	197	26.99	218	29.9	315	43.2
		0.5X	920	52	5.7	20.4	200	26.7	210	28.0	340	45.3
		X	945	58	6.1	21.0	180	23.7	226	29.7	354	46.6
		2X										
<b>K<sub>2</sub>O</b>												
	12h	0.5X	650	92	14.2	14.4	218	33.5	175	26.9	257	39.5
		X	680	110	16.2	15.1	254	37.4	162	23.8	264	38.8
		2X	690	115	16.7	15.3	268	38.8	140	20.3	282	40.9
	24h		620	102	16.5	13.8	210	33.9	145	23.4	265	42.7
		0.5X	650	114	17.5	14.4	232	35.7	120	18.5	298	45.8
		X	640	117	18.3	14.2	262	40.9	110	17.2	268	41.9
		2X										
	36h	0.5X	610	114	18.6	13.5	254	41.6	112	18.4	244	40.0
		X	590	117	19.8	13.1	260	44.1	92	15.6	238	40.3
		2X	580	118	20.3	12.9	246	42.4	100	17.2	234	40.3
<b>Tomaset</b>												
	12h	0.5X	480	120	25.1	10.7	241	50.2	125	26.04	114	23.8
		X	460	125	27.2	10.2	265	57.6	97	21.1	98	21.3
		2X	445	140	31.5	9.9	282	63.4	71	16.0	92	20.7
	24h	0.5X	425	148	34.8	9.4	302	71.1	63	14.8	60	14.1
		X	389	156	40.1	8.6	298	76.6	48	12.3	51	13.1
		2X	360	169	46.9	8.0	285	79.2	33	9.2	42	11.7
	36h		348	182	52.3	7.7	272	78.2	42	12.1	34	9.8
			330	195	59.1	7.3	261	79.3	48	14.5	20	6.1
			308	198	64.3	6.8	256	83.1	38	12.3	14	4.5

the lowest concentration (0.5 X) and disappeared in plants treated with moderate and high concentrations.

With the use of K<sub>2</sub>O six polypeptide having molecular mass of 118.8, 48.93, 47.3, 44.22, 30.6 and 13.3 kDa were disappeared in all concentrations, since they were found in control. At the same time a new band with 14.5 kDa was recognized in the plants treated with the three concentrations and not found in control, also a small band with 95.1 kDa was found with the use of the low and moderate concentrations and two more bands with 39.7 kDa and 22.04 kDa were recognized in plants treated with the lowest, moderate and the highest concentrations, respectively.

On the other hand, the use of GA<sub>3</sub> did not show significant decrease in the protein band numbers specially with the low and moderate concentrations, while the number of protein bands changed from 25 in control to 24 in the plants treated with low and moderate concentrations and to 21 bands with the use of high concentration (2 X). Only one small new band with Mw. 128.97 kDa was de novo synthesized with the use of the three concentrations and two more small bands (95.1 & 12.3 kDa) were synthesized with the use of the lowest concentration only. However three bands with molecular mass of 53.98, 47.30 and 30.60 kDa

disappeared with the use of the high concentration (2 X) and one 44.22 kDa band disappeared with the use of high and moderate concentrations. However, two 110.5 and 13.4 kDa disappeared with the use of high and low concentrations while one 48.93 kDa band was not observed in plants treated with low and moderate concentrations.

## DISCUSSION

The present study showed that the use of the agricultural chemicals GA<sub>3</sub>, K<sub>2</sub>O and Tomaset varies in their effect on alfalfa root tips. The mutagenic potentiality of the Tomaset was obviously greater than the other two chemicals. The chromosomal aberrations induced by the three chemicals used in the present study are similar to aberrations induced by many other chemicals, pesticides, insecticides, chemical mutagens and radiations. These chromosomal aberrations may consider as indicators of clastogenic effects of their inducers (Badr, 1983). The percentage of chromosomal aberrations increased with increase of the concentration of the chemicals applied and as the period of the treatment was prolonged (Table I).

This may indicate the increased of the impairment of

**Table II. Changes in molecular weight (Mol. Wt) and mobility rate (Rm) induced in the alfalfa seed protein components after different treatments with gibberilic acid, potassium oxide and Tomaset**

L. no	Molecular	Control			K <sub>2</sub> O			G A <sub>3</sub>			Tomaset		Mol. Wt	Rm
B. no	Marker	0.5X	X	2X	0.5X	X	2X	0.5X	X	2X	X	2X		
1		2.44	1.73	2.68	2.03	1.21	1.56	2.52	2.32	0	1.10	157.50	0.020	
2		1.68	0.13	1.06	0.12	0.84	1.48	1.66	1.67	0	0	142.14	0.045	
3		0	0.33	0.73	1.39	1.55	1.18	2.70	3.67	0	0	128.97	0.066	
4		8.87	0	0	0	10.50	1.66	8.05	11.00	5.02	0	118.80	0.86	
5		2.95	1.83	0.70	2.30	0	2.34	0	0	5.40	2.33	110.50	0.10	
6		0.81	1.90	1.42	1.25	2.51	8.35	1.89	0	0	0	101.99	0.12	
7	7.91	0	0.72	0.26	0	0.78	0	1.14	0.39	0	0	95.10	0.14	
8		1.46	0.59	0.40	2.38	0.07	0.63	2.42	2.74	0.06	0	84.83	0.17	
9		1.44	1.93	2.66	3.02	0.13	0.41	3.67	1.8	0.36	0	77.35	0.19	
10	12.10	3.52	3.09	2.85	4.35	0.92	0.94	2.88	3.47	2.21	0.92	67.00	0.23	
11		3.67	0	2.93	0	3.54	2.64	4.93	5.00	0	2.02	65.70	0.24	
12		7.62	5.69	3.75	8.13	5.00	5.35	13.80	9.80	3.04	8.87	57.25	0.30	
13		6.99	6.21	5.07	8.91	5.52	5.56	0	10	6.46	4.40	53.98	0.32	
14		4.07	0	0	0	0	0	6.12	0	0	0	48.93	0.37	
15		6.09	0	0	0	4.50	5.07	0	0	4.82	4.02	47.30	0.38	
16		2.45	8.85	9.94	6.25	5.15	8.18	10.9	6.12	11.3	4.26	46.00	0.40	
17		2.62	0	0	0	5.82	0	0	0	0	0	44.22	0.41	
18	18.40	2.38	4.56	0	4.46	6.33	5.46	3.82	4.05	6.30	0	41.25	0.44	
19		0	0	0	0	0	0	0	9.71	8.67	10.80	39.70	0.45	
20		4.10	6.07	2.94	4.32	6.30	3.67	3.00	4.82	8.69	0	37.40	0.48	
21		1.79	1.17	2.34	3.72	3.77	3.16	1.93	2.34	0	17.80	34.22	0.51	
22	12.70	1.57	0	0	0	3.62	4.13	0	1.86	5.46	0	30.60	0.54	
23		2.19	0.85	3.33	2.15	0	3.61	0.75	2.39	5.12	5.63	27.22	0.60	
24		3.70	7.28	6.33	4.65	6.14	7.48	2.80	4.81	0.78	7.25	25.86	0.62	
25		5.17	16.50	14.60	7.32	7.55	9.74	1.09	0.46	0	0.81	23.20	0.67	
26		0	7.23	0	6.74	0	0	0	0	0	0	22.04	0.69	
27	27.20	0.83	9.08	5.13	5.46	0.83	0.32	1.66	0.02	0.01	0.27	20.5	0.72	
28		0.03	0.04	0	0	0.03	0.04	0.01	0	0	0	18.2	0.77	
29		0	0	0	0	0	0	0	0	0	0.30	15.46	0.85	
30	21.20	0	0.40	0.17	0.28	0	0	0	0	0	0	14.5	0.89	
31		0.13	0	0	0	0	0.11	0	0	0	0	13.4	0.9	
32		0	0	0	0	0.26	0	0	0	0	0	12.3	0.95	
Total no.	6	25	22	20	20	24	24	21	21	16	15			

mitotic apparatus, which was not completely inhibited. It is clear from our results that chromosomal stickiness is the most dominant abnormality produced in different treatments. Stickiness is a common physiological phenomenon, which may be the result of an action by the chemicals on chromatin fibers (Badr *et al.*, 1987). It has been attributed to an action on the protein of chromosomes (El-Sadek, 1972). Chromosomal bridges may be due to chromosomal stickiness and subsequent failure of free anaphase separation or may be attributed to un-equal translocation of chromosome segments (Najjar & Soliman, 1980).

In the present study increase in the number of prophase nuclei after treatment with Tomaset was accompanied with decrease in the number of metaphase, anaphase and telophase nuclei. This accumulation of prophases indicates a delay in the spindle formation. These observations were in accordance with the results obtained in *Allium cepa* root tips after using aliphatic alcohols and fatty alcohols (Shehab, 1980).

The results obtained in this study showed a large variation in the number and intensity of polypeptide bands among the different treatments (Fig. 2 & Table II). Total number of polypeptide bands varies from 25 in control plants to 15 bands in seeds treated with 2X concentration of

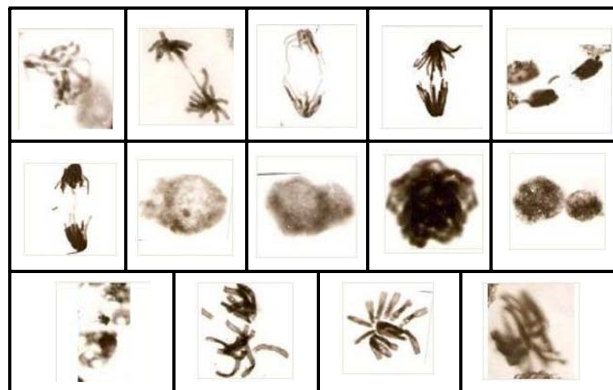
Tomaset. Use of GA<sub>3</sub> caused qualitative and quantitative variation in polypeptide bands but didn't cause complete repression of any protein found in control from the three concentrations (Table II). Some bands disappeared after treatment with low, moderate or high concentration only. This result agrees with (Reyes *et al.*, 2006) who found that some proteins disappear with the treatment by some concentrations of GA<sub>3</sub>.

A specific polypeptide 48.93 kDa band was observed only in plants treated with 2X GA<sub>3</sub>, which was observed in any other treatment, a similar 49.3 kDa protein band was induced in loquat plant when treated with GA<sub>3</sub> (El-Dengawy, 2005). It may indicate that this protein has a specific role in improving growth. El-Dengawy (2005) recommended 250 ppm (2X) GA<sub>3</sub> for the best germination and improving growth characteristics of the subsequent seedlings of *Gentiana lutea* L. and induce different new protein bands. On the other hand, treatment with K<sub>2</sub>O led to complete disappear of 6 polypeptide bands, which were otherwise evident observed in control. This may be due to its toxic and corrosive pollution effect on tissues, in decreasing in the total protein content of *Zinnia elegans* pollen grains (Chehregani *et al.*, 2004).

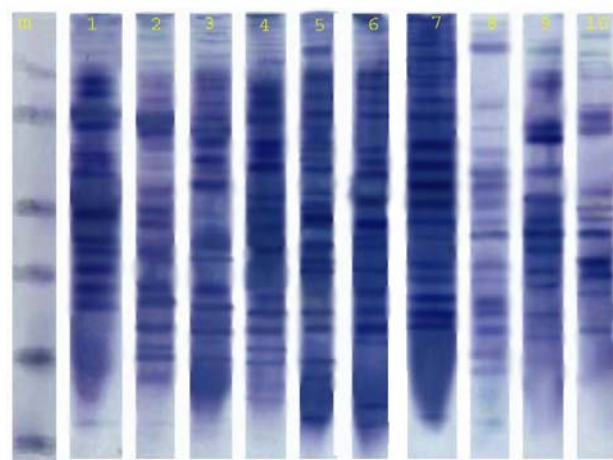
Also a novel 128.97 kDa band was induced after the

**Fig. 1. Abnormalities produced by various treatments of potassium oxide (K<sub>2</sub>O) and Tomaset**

- a: disturbed multipolar anaphase  
 b: star- anaphase with bridge  
 c& d: multibridges anaphase  
 e&f: sticky anaphase with chromosome fragment  
 g&h: deforming interphase nuclei with buds  
 i: sticky prophase  
 j: unequal dividing nucleus  
 k: nuclei vacuolation  
 l: chromosome fragment at metaphase  
 m: star metaphase  
 n: sticky metaphase

**Fig. 2. Electrophoretic pattern of seed protein bands of *Vicia faba* plants treated with the three agriculture chemicals K<sub>2</sub>O, GA<sub>3</sub> and Tomaset and control**

- m: represent mol. wt marker  
 1: control (H<sub>2</sub>O)  
 (2, 3, & 4): seeds treated with x/2, 1x & 2x concentration of K<sub>2</sub>O.  
 (5, 6 & 7): seeds treated with x/2, 1x & 2x concentration of GA<sub>3</sub>.  
 (8, 9 & 10): seeds treated with x/2, 1x & 2x concentration of Tomaset



use of the three concentrations as well as with using all concentrations of GA<sub>3</sub> and the lowest concentration of Tomaset. A very light 95.1 KDa band and distinct 22.04 kDa band were observed only in plants treated with 2X K<sub>2</sub>O. This newly induced band might be related to defense responses in plants. Another point of interest is the

occurrence of a very small faint band with Mw 14.5-KDa in the three different concentrations of K<sub>2</sub>O only and not in control and other treatments. Thus, it is likely a unique marker of K<sub>2</sub>O and might represent a homology with potassium oxide.

The use of Tomaset induced specific dark 39.7 kDa polypeptide at all concentrations, which not found in the other treatment or control. Two other high-molecular mass polypeptides 95.10 and 142.14 kDa appeared only at lowest concentration. These newly synthesized proteins may be induced to protect plants from the different harmful effect caused by different concentrations of Tomaset. Four polypeptide bands disappeared at all concentrations, while the total number of protein bands decreased to 15 at the highest concentration (2 X). This decrease in the number of protein bands might be due to the sever formative and side effect of Tomaset on plants recorded by Morgan (1986).

In conclusion all the chemicals produced marked effects on alfalfa root tips. The cytological studies revealed that chromosomal stickiness was the most common abnormality observed in root tips treated with the three chemicals. Tomaset was the most harmful, which caused the highest frequency chromosomal abnormalities and also decrease in the number of polypeptide in the protein banding profile. The plants treated with GA<sub>3</sub> are characterized by a 48.9 kDa polypeptide, but those treated with K<sub>2</sub>O are characterized a 14.5 kDa protein band. A protein band with a 39.7 kDa protein band was found only in plants treated with all concentrations of N-meta-tolyl phthalamic acid. I think that the study gave an impression that all agricultural chemicals have harmful effect with different degree, which may cause widespread alarm.

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