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RESEARCH ARTICLE

Effect of plant growth regulators on protein banding profile of some higher plants in relation to mitotic activity and the total abnormalities.

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Abstract

Effect of maleic hydrazide (MH) which is a growth retardantand benzyladenine (BA) that is a growth regulator on mitotic index, total abnormalities and protein banding profile of two different plant species was investigated.Two speciestreated with MHincluded *Trigonella foenum-graecum* (fenugreek) and *Allium cepa* (onion), while*Nigella sativa* L. (Black seed) and *Allium cepa* (onion) species was treated by BA. Three concentrations of maleic hydrazide and benzyladenine (15, 35 and 55 ppm) were applied for 6 and 48h. The treatments of root tips and germinated seeds of *T. foenum-graecum* and *A. cepa*withMHshowed an inhibitory effect on cell division in root tips of both plants and caused a decrease in their mitotic index values and induced changes in the numbers and intensity of protein bandsas compared with untreated germinated seeds. Our results, also, showed thatthe applied concentrations of BA appeared a promotor effect on cell division in root tips of *N. sativa* and *A. cepa*and caused an increased in their mitotic index values. These increments in the mitotic activity werecorrelated with marked changes in protein profile, included alterations in band intensity, disappearance of some bands and appearance of new other bands of protein banding patterns, as compared with the negative control.Both of MH and BA were found to be more effective in appearance a new bands (6 and 7) than disappearance of some bands for *T. foenum-graecum* and *N. sativa* at 15 ppm for 6h, respectively.For *A. cepa*, the great number of new bands (10 and 6) was induced after treatment of root tips with high concentration of MH (55 ppm) and lower concentration of BA (15 ppm), respectively for 48 hours.Thus, the application of lowest concentration of BA (15 ppm) could be useful in improvement of seed germination and seedling growth with lowest value of chromosomal abnormalities.

Introduction

Various growth regulators, especially growth retardants have been proven to prevent excessive stem elongation and reduce internode length in plants by inhibiting the effect of cell division and enlargement of cell in plants (Sachset al., 1975and Navale *et al.,* 2010). Maleic hydrazide (MH) is an herbicide and is a regulator of the growth of buds in vegetables during storage (Marcano *et al.,* 2004). It is used in agriculture in despite its known effect as a mutagenic and clastogenic agent. Since 1951 MH has been known as an effective chromosome-breaking agent in higher plants (Darlington and McLeish,1951). In early works the inhibitory effects of MH on plant growth were mainly considered to result from the suppression of plant metabolism (inhibition of enzyme activity) and interference of the compound with plant hormones and growth regulators. Numerous experiments performed with various plant species have shown that MH acts as an inhibitor of the synthesis of nucleic acids and proteins. MH is a pyridazine that inhibits the synthesis of nucleic acids and proteins in *Vicia faba* root tip cells (Murin, 1990; De Marco *et al.,* 1992, 1995,1999 and De Marco, 2005).

Plant growth regulators are substances to have fundamental role in the regulation of the life cycle of the plants (Trewavus, 1981). The cytokinins regulate growth and effect on germination rate in a variety of ways in different plants. Following the treatment of the oat seeds with 10 ppm and 100 ppm of BA, seed germination percentage was enhanced and recoded a value of 44% and 57%, respectively, compared to control value of 28% after 15 days (Sharma *et al*., 1976). Cytokinins, N^6-substituted adenine derivatives, are a class of plant hormones that were first identified as cell division promoter factors (Miller *et al*., 1956). Application of exogenous cytokinin to some organs that normally lack this hormone has been shown to induce cell division (Riou-Khamlichi *et al*., 1999). The cytokinins effect not only cell division but also many other aspects of plant growth and developmental processes including seed germination, shoot initiation and growth, apical dominance, senescence and abscission (Mok 1994; Dewitte *et al*., 1999 and Werner *et al*., 2001). Numerous reports assign a stimulatory or inhibitory effect of cytokinins in different development processes such as root growth and branching, control of apical dominance in the shoot, chloroplast development and leaf senescence (Mok, 1994).

There is correlation between chromosomal aberrations and toxic effects of a number of pesticides at proteomic level. The applications of proteomic tools have become popular and powerful for detecting and examining changes in protein composition accurately (Singh *et al*, 1993).Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is most economical simple and extensively used biochemical technique for analysis of genetic structure of germplasm. The relative ease of performing SDS-PAGE along with its wide applications has made it an important analytical technique in many fields of research (Hafez, 2005).Abd-elsalam *et al.,* (1993), Hassan *et al*., (2002) and Atef et al., (2010) used electrophoretic banding patterns of seed storage proteins for monitoring the mutagenic effects of pesticides and other chemicals.

Therefore, this study was planned to examine the effect of maleic hydrazide(growth retardant) and benzyladenine (growth regulator) on protein banding pattern of *Trigonella foenum-graecum*, *Allium cepa* and *Nigella sativa*, germinated seedsin relation to mitotic abnormalities.

Material and Methods

Plant materials

Seeds of three species of onion (*Allium cepa*), fenugreek(*Trigonella foenum-graecum*) and black seed (*Nigella sativa)*) were obtained from the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

Cytological Examination

Ten germinated seeds, with radicle 2-3 cm long, were treated with different concentrations for different times. Control germinated seeds were placed in distilled water. After each treatment, the roots were cut off and immediately fixed in glacial acetic acid: absolute ethyl alcohol (1:3 *v/v*) for overnight. The root tips were stained by using the Feulgen squash technique. At least three slides for each treatment were examined to determine the mitotic index (MI) which was calculated as the percentage of dividing cells to the total number of cells examined. The same slides were analyzed for the percentage and types of chromosomal abnormalities in cells at each mitotic phase as well as non-dividing cells. The data recorded for different treatments were statistically analyzed using t-test for determine significant differences between these treatments.

Seeds germination and extraction of seedling proteins

The seeds were soaked in distilled water for 2 hours then transferred to Petri-dishes containing moistened filter paper till appearance the radicle. The germinated seeds of each tested plants were selected for uniformity in size and shape. Germinated seeds were classified into three groups, the first and second were immersed in suitable amount of each tested concentrations of 15, 35 and 55 ppm of maleic hydrazide and benzyladenine for the different times of 6 and 48 hours, respectively. Third group of germinated seeds were soaked in distilled water for the same period was run with each treatment as the control.

One gram of treated and untreated (control) germinated seeds of onion (*A. cepa*), fenugreek(*T. foenum-graecum*) and black seed (*N. sativa)* was ground to fine powder with pestle and mortar. Ten mg of powered flour was homogenized thoroughly with 400 µl extraction buffer using vortex. The extraction buffer was prepared by dissolving 0.6 g Tris base, 0.2 g Sodium Dodecyl Sulfate (SDS) and 30 g of urea in 50 ml of double distilled water. One ml of β-mercaptoethanol was added and then the solution was diluted to 100 ml with double distilled water.The mix was kept overnight at 4°C and then centrifuged at 13000 rpm for 10 minutes at room temperature (Sigma 3K 18 Bench Top centrifuge).

Electrophoresis of protein using SDS-PAGE

The seedling proteins were analyzed using continuous SDS-PAGE (Owl vertical slab apparatus) method as described by Laemmli (1970). 25 µl of the extracted protein was boiled in a water bath for 3-5 min and loaded on Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) containing 12.5% resolving gel and 4% stacking gel using 3 µl bromophenol blue as tracking dye. Protein samples (20 µl) including loading dye were loaded in the stacking gel. Electrophoresis was carried out under non-reducing conditions in 12.5 % polyacrylamide gel. The assay was carried out by an electric supply of 15 mA for 30 min, and then raised to 25 mA for 5-6 h, using a protein marker (5 µl) (Prism Ultra Protein Ladder) with low molecular weights until tracking dye reached to the bottom of the gel. The gels were destained in solvent composed of 40 ml of methanol, 10 ml glacial acetic acid and 50 ml distilled water. Gels were then stained in 25 ml of Coomassie Brilliant Blue (R-250) for overnight at room temperature. The gels were photographed and the molecular weights of the polypeptide bands were estimated by correlating position of the molecular weight marker standards. The data obtained from the treated germinated seed protein banding patterns for each studied species were subjected to the analysis by using the lab image program. The presence or absence of each of the bands (coded as + and - respectively).

Result

Effect of maleic hydrazide and benzyladenine onmitotic index (MI)

The influence of MH on mitotic activity or mitotic index (MI) is given in **Table 1**. In general, the MI values reduction in the treated roots of *T. foenum-graecum* and *A. cepa* was a dose and time dependent. For each treatment time, the reduction of MI increased with increasing MH concentrations from 15 to 55 ppm. The mitotic index was remarkably reduced in roots treated with 55 ppm for 48 hours to a minimum value of 0.18 ± 0.02 % in *T. foenum-graecum* and to a value of 0.11± 0.02 % in *A. cepa*.

In both test plants the total percentages of abnormalities increased with increasing the concentration of MH and prolonged the treatment time. In *T. foenum-graecum* the highest percentage of abnormalities was 100.0 ± 0.0 % compared with the control value of 2.77 ± 0.03 % which was recorded in roots treated with 55 ppm for 48 h. In roots treated of *A. cepa* with 55 ppm for 48 all divided cells showed abnormalities (100 ± 0.00 %) compared with a control value of 5.88 ± 0.04 %.

The influence of BA on mitotic activity or mitotic index (MI) is given in **Table 2**. In general, the MI values increasing in the treated roots of *N. sativa* and *A. cepa* was a dose and time dependent. For each treatment time, the increasing of MI increased with increasing BA concentrations from 15 to 55 ppm.The mitotic index was remarkably increased in roots treated with 55 ppm for 48 h to a maximum value of 21.22 ± 0.01 % in *N. sativa* and to a value of 18.12 ± 0.01 % in *A. cepa*.

In both tested plants the total percentages of abnormalities increased with increasing the concentration of BA and prolonged the treatment time. In *N. sativa* the highest percentage of abnormalities was 98.76 ± 0.3 % compared with the control value of 2.38 ± 0.0 % which was recorded in roots treated with 55 ppm for 48 hrs. In roots treated of *A. cepa* with 55 ppm for 48hrs. 85.72 ± 0.04 % of all divided cells showed abnormalities compared with a control value of 2.36 ± 0.0 %.

Effect of maleic hydrazide on protein profile of *Trigonella foenum-graecum*

The electrophoretic patterns of protein bands extracted from treated germinated seeds of *T. foenum-graecum*with three different concentrations of maleic hydrazide for 6 and 48 hours compared to untreated germinated seeds are shown in **Figure1**. A total number of 39 polypeptides bands with their molecular weights are given in **Table 3**. The untreated germinated seeds for 6 hoursshowed 14 polypeptide bands of molecular weights ranged from 15 to 70 KD, whereas the maleic hydrazide treated germinated seeds showed a total number of 25 bands (7, 12 and 6 bands) following with the concentrations of 15, 35 and 55 ppm, respectively. Of these 14 bands of untreated germinated seeds (control), bands with molecular weight of 64, 42, 33, 28 and 23 KD were disappeared in treated germinated seeds with concentrations of 15, 35 and 55 ppm of maleic hydrazide. Also, the results showed that bands with molecular weight of 54, 47, 38, 30, 26, 25, 18 and 15 KD were recorded in control and with one treatment for the different concentrations. Moreover, the treatments of the germinated seeds with different concentrations caused the appearance of a total 15 new polypeptide bands as the following: 6, 4 and 5 with 15, 35 and 55 ppm, respectively

Table 1: Frequency of mitotic phases and the percentage of total abnormalities after treatment of*Trigonellafoenum – graecun* L. and *Allium cepa* L. root tips with different concentration of MH for 6 and 48 hours.

Treatments		*Trigonella foenum-graecum* L.				*Allium cepa* L.			
Time (h)	Conc. (ppm)	Total counted cells	Divided cells	MI ± S.E	Total abno. % (X ± S.E.)	Total counted cells	Divided cells	MI ± S.E	Total abno. % (X ± S.E.)
6	Control	1682	166	9.90 ± 0.12	1.2 ± 1.03	1495	112	7.50 ± 0.66	0.89 ± 0.14
	15	1582	96	6.08±0.08*	41.49±1.08**	1445	48	3.39±0.32*	68.75±3.36**
	35	1528	61	4.03±0.12**	38.48±3.26**	1509	16	1.05±0.01**	87.50±1.83**
	55	1391	19	1.38±0.10**	50.10±1.60**	1289	5	0.41±0.02**	100.0±0.0**
48	Control	735	72	9.80 ± 0.14	2.77 ± 0.84	460	34	7.40 ± 0.16	5.88 ± 2.6
	15	1521	28	1.88±0.02**	89.28±1.99**	1370	11	0.80±0.03**	90.90±2.91**
	35	1414	10	0.70±0.03**	90.0±0.00**	1275	6	0.49±0.02**	100.0±0.0**
	55	1266	2	0.18±0.02**	100.0±0.00**	1174	1	0.11±0.02**	100.0±0.0**

Table 2: Frequency of mitotic phases and the percentage of total abnormalities after treatment of*Nigella sativa* L. and *Allium cepa* L. root tips with different concentrations of BA for 6 and 48 hours.

Treatments		*Nigella sativa* L.				*Allium cepa* L.			
Time (h)	Conc. (ppm)	Total counted cells	Divided cells	MI ± S.E	Total abno. % (X ± S.E.)	Total counted cells	Divided cells	MI ± S.E	Total abno. % (X ± S.E.)
6	Control	1630	135	8.29 ± 0.34	1.48 ±1.03	1450	132	9.10 ± 0.07	0.75±0.1
	15	1725	175	10.15±0.04*	30.28 ± 0.1	1566	160	10.22±0.03*	18.75±0.3*
	35	1955	225	11.5±0.03*	40.0±0.03**	1645	188	11.43±0.01*	26.60±0.8*
	55	2120	280	13.20±0.01**	48.92±0.3**	1698	210	12.36±0.02*	39.04±0.5*
48	Control	1530	126	8.23 ± 0.05	2.38 ± 0.0	1340	127	9.32 ± 0.14	2.36±0.6
	15	1788	238	13.31±0.03**	84.03±0.2**	1610	200	12.42±0.04**	66.0±0.2**
	35	2215	380	17.15±0.02**	94.74±0.2**	1715	273	15.92±0.02**	76.92±0.4**
	55	2290	486	21.22±0.01**	98.76±0.3**	1738	315	18.12±0.01**	85.72±0.3**

S.E., Standard error ***** Significant at 5% level ($P \leq 0.05$) ******Significant at 1% level ($P \leq 0.01$)

The treatments for 48 hours, a total number of 23 protein bands were observed. The untreated germinated seeds of *T. foenum-graecum*showed 3 polypeptide bands of the molecular weights ranged from 40 to 65 KD, whereas the germinated seeds showed the total number of bands of (20 bands) as 3, 5 and 12 bands after the treatment with the concentrations of 15, 35 and 55 ppm of MH, respectively. The 3 bands of untreated germinated seeds (control) with molecular weight of 65, 59 and 40 KD were also recorded with one treatment for the different concentrations. Moreover, the treatments of the germinated seeds with different concentrations caused the appearance of a total of 17 new polypeptide bands as 3, 4 and 10 with 15, 35 and 55 ppm, respectively. The band with molecular weight 48 KD was recorded with two treatments of 35 and 55 ppm similar to the band with molecular weight of 41 which was recorded with two different treatments of 15 and 35 ppm.

Effect of maleic hydrazide on protein profile of *Allium cepa*

The SDS-PAG electrophoretic patterns of polypeptide bands in the untreated and treated germinated seeds of *A. cepa* for 6 and 48 hours with different concentration of maleic hydrazide (15, 35 and 55 ppm) are given in **Figure1**. Scanning analysis of electropherogram for molecular weights of total bands 37 for 6 hours and 29 bands for 48 hours is tabulated in **Table 4**. The results for 6 hours showed that the appearance of 13 bands of polypeptides (molecular weights ranged from 19 KD to 62 KD) in untreated germinated seeds (control) and (a total 24bands) 8, 8 and 8 bands in germinated seeds treated with 15, 35 and 55 ppm of maleic hydrazide, respectively. Of these 13 bands, 7 bands with molecular weights of 62, 58, 51, 48, 42, 35 and 32 were disappeared in the treated germinated seeds with the different applied concentrations of maleic hydrazide whereas 5 bands with molecular weight of 44, 39, 30, 28 and 27 were observed in untreated (control) and in treated germinated seeds with all concentrations of the experimental treatments.

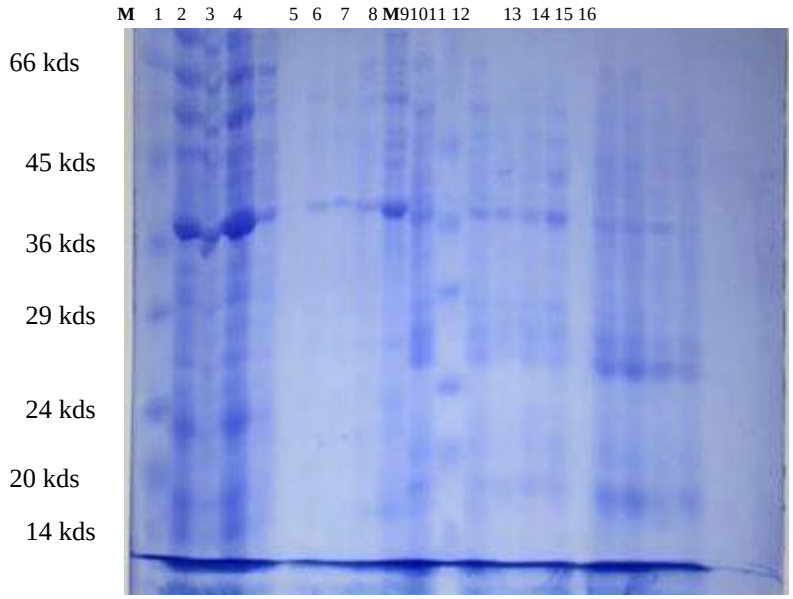

Figure 1: Electrophoretic of protein banding patterns of *Trigonella foenum-graecum* and *Allium cepa* showing the effect of different concentrations of maleic hydrazide (15, 35 and 55 ppm) for 6 and 48 hours. M = Marker. 1= Control of 6 hours treatment of *T. foenum graecum* L.2 =Treatment of *T. foenum graecum* L. with 15 ppm for 6 hours.3 = Treatment of *T. foenum graecum* L. with 35 ppm for 6 hours.4 = Treatment of *T. foenum graecum* L. with 55 ppm for 6 hours.5= Control of 48 hours treatment of *T. foenum graecum* L. 6 = Treatment of *T. foenum graecum* L. with 15 ppm for 48 hours. 7 = Treatment of *T. foenum graecum* L. with 35 ppm for 48 hours. 8 = Treatment of *T. foenum graecum* L. with 55 ppm for 48 hours.9 = Control of 6 hours treatment of *A. cepa* L.10= Treatment of *A. cepa* L. with 15 ppm for 6 hours. 11= Treatment of *A. cepa* L. with 35 ppm for 6 hours. 12 = Treatment of *A. cepa* L. with 55 ppm for 6 hours. 13 = Control of 48 hours treatment of *A. cepa* L. 14 = Treatment of *A. cepa* L. with 15 ppm for 48 hours. 15 = Treatment of *A. cepa* L. with 35 ppm for 48 hours. 16 = Treatment of *A. cepa* L. with 55 ppm for 48 hours.

Table 3: Effect of maleic hydrazide on protein profile of *Trigonella foenum-graecum* after treatment of germinated seeds with (15, 35 and 55 ppm) for 6 and 48 hours. (+) = presence of band and (-) = Absence of band.

No.	M.W (kd)	Marker	Treatments for 6 hr.				Treatments for 48 hr.			
			Control 1	15 ppm 2	35 ppm 3	55 ppm 4	Control 5	15 ppm 6	35 ppm 7	55 ppm 8
1	72	-	-	-	-	-	-	-	-	+
2	70	-	+	-	+	+	-	-	-	-
3	68	-	-	+	-	-	-	-	-	-
4	66	+	-	-	-	+	-	-	-	-
5	65	-	-	-	+	-	+	-	-	+
6	64	-	+	-	-	-	-	-	-	-
7	63	-	-	-	-	-	-	-	+	-
8	62	-	-	+	-	-	-	-	-	-
9	60	-	-	-	-	-	-	-	-	+
10	59	-	-	-	-	-	+	-	+	-
11	58	-	-	-	-	-	-	-	-	-
12	57	-	-	-	-	-	-	+	-	-
13	56	-	-	-	-	+	-	-	-	-
14	54	-	+	-	+	-	-	-	-	-
15	51	-	-	+	-	-	-	-	-	+
16	49	-	-	-	-	+	-	-	-	-
17	48	-	-	-	-	-	-	-	+	+
18	47	-	+	-	+	-	-	-	-	-
19	45	+	-	-	-	-	-	+	-	-
20	44	-	-	-	-	-	-	-	-	+
21	43	-	-	-	-	-	-	-	+	-
22	42	-	+	-	-	-	-	-	-	-
23	41	-	-	-	-	-	-	+	+	-
24	40	-	-	-	-	-	+	-	-	+
25	39	-	-	-	-	+	-	-	-	-
26	38	-	+	-	+	-	-	-	-	-
27	37	-	-	+	-	-	-	-	-	-
28	36	+	-	-	-	-	-	-	-	-
29	34	-	-	-	+	-	-	-	-	-
30	33	-	+	-	-	-	-	-	-	-
31	31	-	-	-	-	+	-	-	-	-
32	30	-	+	-	+	-	-	-	-	-
33	29	+	-	+	-	-	-	-	-	+
34	28	-	+	-	-	-	-	-	-	-
35	27	-	-	-	+	-	-	-	-	+
36	26	-	+	+	-	-	-	-	-	-
37	25	-	+	-	+	-	-	-	-	+
38	24	+	-	-	+	-	-	-	-	-
39	23	-	+	-	-	-	-	-	-	+
40	20	+	-	-	-	-	-	-	-	-
41	18	-	+	-	+	-	-	-	-	-
42	17	-	-	+	-	-	-	-	-	+
43	15	-	+	-	+	-	-	-	-	-
44	14	+	-	-	-	-	-	-	-	-
Total bands		7	14	7	12	6	3	3	5	12
Disappearedbands			0	5	5	5	0	-	-	-
New bands			0	6	4	5	0	3	4	10

Table 4: Effect of maleic hydrazide on protein profile of *Allium cepa* L. after treatment of germinated seeds with (15, 35 and 55 ppm) for 6 and 48 hours.(+) = presence of band and (-) = Absence of band.

No.	M.W (kd)	Marker	Treatments for 6 hr.				Treatments for 48 hr.			
			Control 9	15 ppm 10	35ppm 11	55ppm 12	Control 13	15ppm 14	35ppm 15	55ppm 16
1	66	+	-	-	-	-	-	-	-	-
2	62	-	+	-	-	-	-	-	-	-
3	59	-	-	-	-	-	+	-	-	-
4	58	-	+	-	-	-	-	-	-	-
5	57	-	-	+	-	-	-	-	-	-
6	56	-	-	-	+	+	-	-	-	-
7	53	-	-	-	-	-	+	-	-	-
8	51	-	+	-	-	-	-	-	-	-
9	49	-	-	+	+	+	-	-	-	-
10	48	-	+	-	-	-	-	-	-	-
11	45	+	-	-	-	-	+	-	-	-
12	44	-	+	+	+	+	-	+	+	-
13	43	-	-	-	-	-	+	-	-	-
14	42	-	+	-	-	-	-	-	-	-
15	41	-	-	-	-	-	+	+	+	-
16	39	-	+	+	+	+	-	-	-	-
17	38	-	-	-	-	-	+	+	+	-
18	36	+	-	-	-	-	-	-	-	-
19	35	-	+	-	-	-	-	-	-	-
20	32	-	+	-	-	-	-	-	-	-
21	30	-	+	+	+	+	-	-	-	-
22	29	+	-	-	-	-	+	+	-	-
23	28	-	+	+	+	+	+	-	-	-
24	27	-	+	+	+	+	-	+	+	+
25	26	-	-	-	-	-	+	+	+	+
26	25	-	-	-	-	-	-	+	-	-
27	24	+	-	-	-	-	-	-	-	-
28	22	-	-	-	-	-	-	-	-	-
29	21	-	-	-	-	-	+	+	-	-
30	20	+	-	+	+	-	-	-	-	-
31	19	-	+	-	-	+	-	-	-	-
32	18	-	-	-	-	-	+	+	+	+
33	14	+	-	-	-	-	-	-	-	-
Total bands		7	13	8	8	8	11	9	6	3
Disappeared bands			0	7	7	7	0	5	5	5
New bands			0	3	3	2	0	3	2	1

The results showed that the band with molecular weight of 19 KD was detected only 55 ppm treatment in addition to control. 8 bands with the molecular weight of 57 (one treatment), 56 (two treatments), 49 (three treatments) and 20 (two treatments) KD were also scored. The bands with 57, 49 and 20 KD were observed with the 15 ppm of maleic hydrazide. The bands with 56, 49 and 20 KD were detected with the 35 ppm and the bands with 56 and 49 were also detected with the treatment of 55 ppm of maleic hydrazide. The treatments for 48 hours, the untreated germinated seeds showed 11 polypeptide bands of the molecular weights ranged from 18 to 59 KD, whereas the germinated seeds treated with the concentrations of 15, 35 and 55 ppm of maleic hydrazide showed (a total number of bands of 18 bands) 9, 6 and 3 bands, respectively. Of these 11 bands, 5 bands with 59, 53, 45, 43 and 28 were disappeared in the treated germinated seeds with the different applied concentrations of maleic hydrazide. In treated and untreated germinated seeds, two bands with 26 and 18 KD were recorded with all applied concentrations. Also, two bands with molecular weight of 41 and 38 KD were recorded with 2 different concentrations (15 and 35 ppm). In this

respect, two bands with 29 and 21 KD were recorded with one treatment (15 ppm). Moreover, the treatments of the germinated seeds with different concentrations caused an appearance of a total 6 new polypeptide bands 3, 2 and 1 with 15, 35 and 55 ppm, respectively. The band with 44 KD was recorded with two treatments of 15 and 35 ppm in contrast to the band with molecular weight of 27 which was recorded with all treatments. The band molecular weight of 25 KD was only scored with the treatment of 15 ppm of maleic hydrazide.

Effect of benzyladenine on protein banding pattern of *Nigella sativa*

The results of the SDS-PAG electrophoretic patterns of protein bands extracted from untreated and treated germinated seeds of *N. sativa* with different concentrations of benzyladenine (15, 35 and 55 ppm) for 6 and 48 hours are represented in **Figure 2**. A total number of 26 and 16 bands for 6 and 48 hours were recorded in treated and untreated germinated seeds and are given in **Table 5**. From these results, it can be seen that 5 polypeptide bands appeared in control (untreated germinated seeds) whereas (a total 21 bands) 10, 6 and 5 bands were detected in the treated germinated seeds for 6 hours with the concentrations of 15, 35 and 55 ppm of benzyladenine, respectively. Of these 5 bands of proteins in control, thebands with 44 and 17 KD were disappeared from the treated materials, whereas band with molecular weight of 25 was recorded with 2 different concentrations (15 and 35 ppm).Each of bands with molecular weight of 31 and 15 was detected with all concentrations. The treatments induced a new13 bands only in the treated germinated seeds: 7 bands with 15 ppm, 3 bands with 35 ppm and 3 bands with 55 ppm.

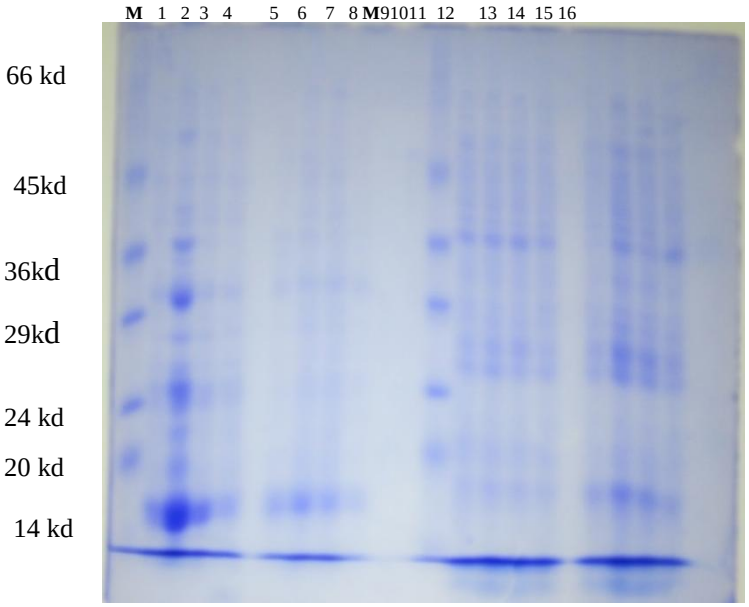

Figure 2: Electrophoretic of protein banding patterns of *Nigella sativa* L. and *Allium cepa* L. showing the effect different concentrations of benzyladenine (15, 35 and 55 ppm) for 6 and 48 hours. M= Marker. 1= Control of 6 hours treatment of *N. sativa*.2 =Treatment of *N. sativa* L. with 15 ppm for6 hours.3 = Treatment of *N. sativa* with 35 ppm for 6 hours. 4 = Treatment of *N. sativa* L. with 55 ppm for 6 hours. 5 = Control of 48 hours treatment of *N. sativa*. 6 = Treatment of *N. sativa* L. with 15 ppm for 48 hours. 7 = Treatment of *N. sativa* L. with 35 ppm for 48 hours. 8 = Treatment of *N. sativa* L. with 55 ppm for 48 hours. 9 = Control of 6 hours treatment of *A. cepa*. 10 = Treatment of *A. cepa* L. with 15 ppm for 6 hours. 11= Treatment of *A. cepa* L. with 35 ppm for 6 hours. 12 = Treatment of *A. cepa* L. with 55 ppm for 6 hours. 13 = Control of 48 hours treatment of *A. cepa*. 14 = Treatment of *A. cepa* L. with 15 ppm for 48 hours. 15 = Treatment of *A. cepa* L. with 35 ppm for 48 hours. 16 =Treatment of *A. cepa* L. with 55 ppm for 48 hours.

For 48 hours treatments, the results in Table (3) revealed that a total 16 protein bands. In untreated germinated seeds, 4 bands of polypeptides with molecular weights of 31, 25, 20 and 16 KD were detected. Of these bands, only the band with molecular weight of 20 KD was disappear in the protein profile following the treatment of germinated seeds. Two of these 4 bands (25 and 16 KD) were scored in treated germinated seed with all concentrations. The fourth band (31 KD) of control was also recoded with the treatment of 55 ppm. Moreover, the rest of 16 bands (in

treated germinated seeds) was recorded a new bands and was only detected following the treatments with 15 and 35 ppm of benzyladenine.

Effect of benzyladenine on protein banding pattern of *Allium cepa*

The results of SDS-PAGE electrophoretic patterns of protein bands extracted from treated and untreated germinated seeds of *A. cepa* are represented in **Figure 2**. For 6 hours treatments, in total of 29 and 27 bands in untreated and treated germinated seeds for 6 and 48 hours, respectively are given in **Table 6**. From these results, 8 polypeptides bands of the molecular weights ranged from 17 to 61 KD were observed in untreated materials whereas the germinated seeds treated with benzyladenine showed the total number of bands of (21 bands) 7, 7 and 7 bands with the concentrations of 15, 35 and 55 ppm, respectively.

Table 5: Effect of benzyladenine on protein profile of *Nigella sativa* L. after treatment of germinated seeds with (15, 35 and 55 ppm) for 6 and 48 hours.(+) = presence of band and (-) = Absence of band.

No.	M.W (kd)	Marker	Treatments for 6 hr.				Treatments for 48 hr.			
			Control 1	15 ppm 2	35ppm 3	55ppm 4	Control 5	15ppm 6	35ppm 7	55ppm 8
1	**66**	+	-	-	-	-	-	-	-	-
2	58	-	-	-	-	-	-	+	-	-
3	52	-	-	+	+	-	-	-	-	-
4	**45**	+	-	-	-	-	-	-	-	-
5	44	-	+	-	-	-	-	-	-	-
6	43	-	-	-	-	-	-	-	-	-
7	42	-	-	-	-	+	-	-	-	-
8	41	-	-	+	+	-	-	-	-	-
9	40	-	-	-	-	-	-	-	-	-
10	39	-	-	+	-	-	-	-	-	-
11	38	-	-	-	-	-	-	-	-	-
12	37	-	-	+	+	+	-	+	+	-
13	**36**	+	-	-	-	-	-	-	-	-
14	34	-	-	-	-	-	-	-	-	-
15	32	-	-	-	-	-	-	+	+	-
16	31	-	+	+	+	+	+	-	-	+
17	**29**	+	-	-	-	-	-	-	-	-
18	28	-	-	+	-	+	-	-	-	-
19	26	-	-	-	-	-	-	-	-	-
20	25	-	+	+	+	-	+	+	+	+
21	**24**	+	-	-	-	-	-	-	-	-
22	23	-	-	+	-	-	-	-	-	-
23	21	-	-	-	-	-	-	-	-	-
24	**20**	+	-	-	-	-	+	-	-	-
25	19	-	-	+	-	-	-	-	-	-
26	17	-	+	-	-	-	-	-	-	-
27	16	-	-	-	-	-	+	+	+	+
28	15	-	+	+	+	+	-	-	-	-
29	**14**	+	-	-	-	-	-	-	-	-
Total bands		7	5	10	6	5	4	5	4	3
Disappeared bands			0	2	2	2	0	1	1	1
New bands			0	7	3	3	0	3	2	0

All detected bands in control were observed in treated germinated seeds. Of these bands, three bands were recorded in all treatments, one band with two treatments and 4 bands with one different treatment. Moreover, the results showed that a new 6 bands was only detected following the treatments with 15 ppm (1 band), 35 ppm (2 bands) and 55 ppm (3 bands).

For 48 hours treatments, in total of 27 bands in untreated and treated germinated seeds with benzyleadenine are given in Table (4). From these results, 5 polypeptides bands of the molecular weights ranged from 17 to 51 KD were observed in control whereas the germinated seeds treated with benzyladenine showed the total number of bands of (22 bands) 10, 7 and 5 bands with the concentrations of 15, 35 and 55 ppm, respectively. All detected bands in control were observed in treated germinated seeds except the band with molecular weight of 51 KD.Two bands were recorded in all treatments and 2 bands with different molecular weight in the same treatment. In addition to the above results, a new 14 bands with different molecular weights was only detected following the treatments with 15 ppm (6 band ranged from 24 to 60 KD), 35 ppm (5 bands ranged from 16 to 49 KD) and 55 ppm (3 bands ranged from 16 to 49 KD).

Table6: Effect of benzyladenine on protein profile of *Allium cepa* L. after treatment of germinated seeds with (15, 35 and 55 ppm) for 6 and 48 hours. (+) = presence of band and (-) = Absence of band.

No.	M.W (kd)	Marker	Treatments for 6 hr.				Treatments for 48 hr.			
			Control 9	15 ppm 10	35ppm 11	55ppm 12	Control 13	15ppm 14	35ppm 15	55ppm 16
1	66	+	-	-	-	-	-	-	-	-
2	61	-	+	-	+	-	-	-	-	-
3	60	-	-	-	-	+	-	+	-	-
4	52	-	+	-	+	-	-	-	-	-
5	51	-	-	+	-	-	+	-	-	-
6	50	-	-	-	-	+	-	-	-	-
7	49	-	-	-	-	-	-	+	+	+
8	45	+	+	+	-	-	-	-	-	-
9	41	-	-	-	-	-	-	+	-	-
10	38	-	+	+	-	-	-	-	-	-
11	37	-	-	-	+	+	+	+	-	-
12	36	+	-	-	-	-	-	-	+	+
13	32	-	-	-	-	-	-	+	-	-
14	29	+	-	-	-	-	-	-	-	-
15	27	-	+	+	+	+	+	+	+	+
16	26	-	+	+	+	+	+	+	+	+
17	25	-	-	-	-	-	-	+	+	-
18	24	+	-	-	-	-	-	+	+	-
19	20	+	+	+	+	+	-	-	-	-
20	17	-	+	+	-	-	+	+	-	-
21	16	-	-	-	+	+	-	-	+	+
22	14	+	-	-	-	-	-	-	-	-
Total bands		7	8	7	7	7	5	10	7	5
Disappeared bands			0	0	0	0	0	1	1	1
New bands			0	1	2	3	0	6	5	3

Discussion

Results in this study showed that theherbicide maleic hydrazide induced different mitotic changes in root tip cells of *Trigonella foenum-graecum* and *Allium cepa*. Such changes vary from (i) reduction of mitotic index of meristematic cells, (ii) changes in phase index and (iii) production of a large number of chromosomal aberrations. These changes appeared in varying degrees depending on duration of the treatment, where the maleic hydrazide caused gradually decreased in mitotic index, when compared with their respective controls. The degree of mitotic inhibition is clearly dose dependent. Similar to our results, the reduction of mitotic activity seems to be a common effect of MH on mitosis in different plants as was mentioned by many investigators (Cortes *et al*., 1985; Patil and Bhat, 1992; Edwin and Reddy, 1993; Osiecka and Janas, 1998, Mendhulkar, 2000; Mohantty *et al*., 2003; Kaymak, 2005; Sobita and Bhagirath, 2005; Juchimiuk, *et al*., 2007; Jabee, *et al*., 2008; Wiszniowski *et al.*, 2009; Pérez *et al*., 2011; Siddiqui *et al*., 2012). The results in this investigation showed that the MH has a significant effect on the total percentages of the abnormalities induced in root tip cells in both plants and it was found to be positively correlated with the

concentration and treatment time and was apparently different from that of the control. Chromosomal abnormalities constitute a significant portion of genetic damage produced by most mutagenic agents (Kaymak, 2005).The total percentages of the abnormalities increased gradually with increase the MH concentration and as the period of treatment was prolonged as reported by (Sabale and Mane, 2000 and Marcano *et al*., 2004). In general, the total percentages of mitotic aberrations induced by MHin *A. cepa* - root tip cells were more than that induced in *T. foenum-graecum*. Similar observations of induction of chromosomal abnormalities have earlier been recorded by many workers tested the effect maleic hydrazide on different plants root tip cells such as (Osiecka and Janas, 1998; Sabale and Mane, 2000; Miadoková *et al.,* 2001; Gichner., 2003; Marcano *et al*., 2004; Kaymak, 2005; Sobita and Bhagirath, 2005; Juchimiuk *et al*., 2006; Jabee *et al*., 2008; Debenest *et al*., 2008; Ghosh *et al.,* 2010; and Siddiqui *et al*., 2012).

In present investigation, the response of mitotic activity affecting root growth of *A. cepa* (onion) and *N.sativa* (black seed plant) to BA was studied with time and dose depended experiments. It is known that plant growth regulators have positive or negative effects on cell division (Tabur and Demir, 2010). Similarly, seed treatments with benzyladenine have enhanced mitotic activity in various field crops and vegetables as previously reported by several investigators (Reyes *et al*., 1991; Soh and Yang, 1993 (at lower concentrations); Wismer*et al*., 1995; Riou-Khamlichi *et al*., 1999; Temmerman *et al.,* 2001;Falkhutdinova *et al*., 2002; Cristea *et al*., 2008 and Huyluoglu *et al*., 2008). The number of cells may be increased in three ways: (1) by more rapid cell division during the cell division phases of fruit growth, (2) by extended cell division for a longer period than normal, or (3) by some combination of these two phenomena (Wismer *et al*., 1995). The results showed that the BA has a significant effect on the total percentages of the abnormalities induced in root tip cells in both plants and it was found to be positively correlated with the concentration and treatment time and was apparently different from that of the control. The total percentages of the abnormalities increased gradually with increase the BA concentration and as the period of treatment were prolonged. In general, the total percentages of the mitotic aberrations induced by BA in *N. sativa* was more than that in *A. cepa* L. Similar observations of induction of chromosomal abnormalities have earlier been recorded by many workers testedthe effect of benzyladenine on different plants root tip cells such as (Pavlica *et al.,*1992; Soh and Yang, 1993;Shepherd and Dos Santos, 1996; Werner *et al.,* 2001; Cristea *et al.*, 2008; Huyluoglu *et al*., 2008; Tabur and Demir, 2010 and Giménez-Alvarado and Colmenares-Esqueda, 2011). In contrast to the above mentioned results, the 100 μM of BA for 24 hours did not induced any chromosomal abnormalities in root tip cells of barley plant (Tabur and Demir, 2010). In this connection, they concluded that effects of plant growth regular on this parameter depend on the plant species, concentration levels and methods of pretreatment.

In our investigation the response of the tested different plants to the treatment was differed. The maleic hydrazide was more effective in appearance of more new bands in treated seeds of the tow tested plants more than disappearance of some bands with the tow treatment time (*T. foenum graecum* and *A. cepa)*. In similar, benzyladenine was less effective in reducing the protein band in *N. sativa* and *A. cepa* and more effective in appearance of new bands in both treatment times. The treatment of 6 hours of *A. cepa* with maleic hydrazide and *N. sativa* with benzyladenine was more effective in inducing new bands than 48 hours. In contrast, the treatment for 48 hours of *A. cepa* with benzyladenine and *T. foenum-graecum* with maleic hydrazide was less effective in inducing new bands than 6 hours. Electrophoretic analysis of the protein bands, which reflects a separate gene, provides information concerning the structural genes and their regulatory systems thatcontrol the biosynthetic pathways of that protein. The disappearance of electrophoretic bands could be attributed to the loss of the genetic materialsdue to laggards and micronuclei that lead to the loss of thegenetic materials. The presence of laggards and bridges in un-publication data (for authors) support thisconclusion, whereinduction of laggards and bridges by these growth regulators may lead to the loss of genetic materials. Therefore, some electrophoretic bands were disappeared due to the deletion of their corresponding genes (Abdelsalam et al., 1993b and El-Nahas, 2000).The disappearance of some bands in solubleproteins of *T. foenum graecum*,*N. sativa*and*A. cepa* due to treated withMH and BA could be explained, also, on the basis of mutational event at the regulatory genes that prevent or attenuate their transcription process (Muller &Gottschalk, 1973).

The changes in the protein bandingpatterns were pronounced in the high molecular weight region than in the middle and lowerregions. This could be attributed to that the genes encoding protein bands inthis region may be more sensitive to the mutagenicity than the genes encoding protein bands in the other two regions.

The appearance of new bands could be explained on the base of mutational event at the regulatory system of unexpected gene (s) that activated it (Abdel-Salam *et al.,* 1997)). Changes in band intensities could be explained on the basis of induction of gene mutation at the regulator genes that attenuate or increase transcription rate of a particular gene that lead to production of a faint or a deep dark protein bands (Gamal El-Din *et al.,* 1988 and Hassan

1996).Also, the recorded changes in band intensity (percentage) could be attributed to the cytological abnormalities induced by both plant regulators (El-Ghamery *et al*., 2002). In this respect the increase in band intensity could be interpreted on the base of gene duplication which is a result of cytological abnormalities.

Conclusion

Treatment with MH and/or BA revealed adverse effect on mitotic activity and the percentages of the abnormalities for three tested plants and this effect was positively correlated with the concentration and the period of treatment.The total percentages of mitotic aberrations induced by MHin *A.cepa root* tip cells were more than that induced in *T. foenum-graecum* cells.The total percentages of the mitotic aberrations induced by BA in *N. sativa*root tip cells were more than that induced in *A. cepa*.For protein banding profile, the treatment with MH and BAshowed marked changes in the protein banding patterns in relation to mitotic abnormalities. These effects included alterations in band intensity, disappearance of some bands and appearance of new other bands of protein banding patterns, as compared with the negative control.Similar trend was recorded for MH and BA intheireffectiveness in production and formationof new bands in treated seeds of the three tested plants more than disappearance of some bands at the tow times of treatment.Using the lowest concentration of BA could be useful for improvement of seed germination and seedling growth with lowest value of chromosomal abnormalities.

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