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Cytogenetic effects of Naphthalene Acetic Acid and Benzylaminopurine in Meristematic Cells of Onion Roots

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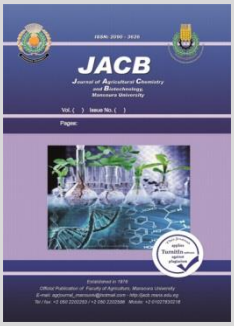

ABSTRACT

This study aimed to investigate the cytogenetic effects of two plant plant growth regulator as, naphthalene acetic acid(NAA)and benzylaminopurine(BAP)in root meristematic cells of onion.The categories of mitotic index rate , as well as, chromosomal aberrations and types of metaphase disturbance were comparatively analyzed after germinated the onion in different concentrations of each hormone.Both hormones stimulated cell division and have aneugenic potential by acting as spindle posion via disturbed the correct separation of chromosomes to the opposite cell poles.The highest percentage of irregular cell shape was induced by five ppm of NAA.The treatments of BAP appeared a dose – response for increasing the percentage of extended cells.All treatments increased the percentage of chromosomal abnormalities above the control group.The most dominant phenotypes of abnormalities were binucleate cells, ghost cells, vacuolated nuclei, necrosis and extended cells, which reflected the toxicity of phytohormones leading to cell death.The most dominant cytological irregularities were c-mitosis, diagonal cells, bridges at ana-telophase, chromosomal breaks and stickness which indicates clastogenic potential of these hormones.Abnormalities induced reflected the action of stimulators on the spindle apparatus such as c-metaphase, laggards chromosomes and multipolar of ana-telophases.All treatments changed the percentage of mitotic index such decreased by NAA or increased by BAP in relation to control.The decline in mitotic index reflected the inhibition of DNA synthesis. Prophase cells declined, meanwhile metaphase and anaphase cells were increased in relation to control.It can be concluded that phytohormones caused toxic effects on the eukaryotic cells via induced different types of irregular mitosis as shown in this study.

Keywords: Phyto - regulators, *Allium cepa*, mitotic abnormalities, mitotic index.

INTRODUCTION

Plant growth hormones plays a significant role in the regulation of physiological processes. They are used to obtained higher crop yields through the regulation of physiological processes. They induced genotoxic effects in living flora as results of accumulated effects in the food to a toxic levels and subsequently affected on the animal and human health (Ateeq *et al*.2002). Phytohormones are widely used in horticulture, floriculture and cell cultures etc. Plant growth regulators induced modification in DNA through the incorporation of labeled thymidine which modify nucleic acid synthesis. Therefore, auxins increases A+T rich fraction, while gibberellic acid increased G+C fraction, meanwhile the cytokinins had the lower effect (Nagl and Rücker 1976). In recent agriculture biotechnology 2,4-D was used in high concentrations to control weed as a herbicide, while it was used in low concentrations as a phyto- regulator. There are several literatures on the genotoxicity of 2,4-D (Kumari and Vaidyanath 1999). It confirm modifications in the cell cycle, changes chromatin and chromosome structure (Pavlica *et al* .1991). Agriculture chemicals as plant growth hormones are the latest trend in crop production (Reddy and Rao 1982). Some of these hormones used at lower doses may induce toxic effects at the higher concentrations. Some of the progressive farmers used phytohormones as insecticides and herbicides such alone or in combinations. These interferes with chromosomal duplication and genome stability which may affect directly or indirectly on the yield and its components (Reddy and Rao 1982). Cytokinins are another class of plant hormones which identified as cell division promoter (El–Ghamery and Mousa 2017). It was affect not only cell division but also many other aspects of developmental processes as seed germination, shoot initiation and apical dominance (Werner *et al*.2001). Phytohormones affected on chromosomal behavior leading to different kinds of abnormalities as oriented metaphase, chromosomal bridges, binucleate cells and micronuclei formation (Huyluoglu *et al*.2008). Phytohormones absorbed by leaves and roots and translocated in the xylem and phloem leading to inhibits cell division in the meristematic location because of chromosomal abnormalities induced (Hartley and Kidd 1987). Several reports assign a stimulatory or inhibitory effect of phytohormones like cytokinins on different developmental processes as root growth, branching, apical dominance of the shoot, chlorophyll formation and leaf senescence (Mok 1994). Plant growth hormones plays an important roles in blocking or slowing the cell cycle progression (Shabbir and Khan 2000). Some of them arrested cells in G_1, whereas others arrested root meristematic cells in G_1 and G_2 phases (Müller *et al*.1994). Thus, Mambelli and Setter (1998) found that cell division was decreased by 50% via the application of abscisic acid (ABA) as a plant growth hormone in maize endosperm. However, Jacqmard *et al*.(1995) explained that abscisic acid slowed DNA replication via inactivating some of DNA replication origins .Cytokinins promoting cytokinesis, mitosis and increasing mitotic activity (Tomaszewska– Sowa *et al* .2002). Gibberellins (GAs) stimulates cell division, cell elongation and mitotic activity (MacDonald and Little 2006). Plants need to produce a huge amounts of chemicals to respond to adapt environmental changes. Phytohormones are one in these prominent positions which represent essential link of many plant processes. Plants respond to biotic or abiotic stresses via

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hormone signal transduction pathways that induced changes in hormonal metabolism and their distribution within the plant (Davies 2004). Genetic toxicology gained much attention in recent years involved in detecting DNA damage and report the mode of action of phytohormones caused alterations in the genome (Uhi *et al*.2003). Stimulators affect morphogenesis by controlling the frequency of cell division and cell cycle (Begum *et al*.1994). These changes often parallel the pattern of DNA synthesis and plant growth (Altamura *et al*.1996). Cytokinin concentrations were highly increased in meristematic locations as roots, young leaves, developing fruits and seeds (Arteca 1996). Application of exogenous cytokinin to some organs that normally defect these hormones had shown to increase the rate of cell division (Riou–Khamlichi *et al*.1999). Gibberellic acid is a fungal product plays a significant role in many cellular processes as promoting stem elongation, fruit setting, seed germination and sex expression (Tuluce and Celik.2006). Kinetin was isolated by miller *et al*. (1995) from yeast extract and DNA of herring sperm and calf thymus. It was found to produce a high rate of cell multiplication in tobacco tissue. The *Allium* test is highly sensitive and good indicators for genotoxicity and carcinogenicity of various agents (Askin Celik and Aslantürk 2010). This test was an excellent model *in vivo*, where roots directly contacted with the test agent enabling possible damage to eukaryotic DNA to be predicted. Therefore, the results from this test can be extrapolated for animal and plant biodiversity (Tedesco and Laughinghouse 2012). *Allium* test has been referred as a standard technique for cytotoxicity screening of plant growth regulators (Nielson and Rank 1994). The results of the *Allium* test may appear cytotoxic or genotoxic effects of plant growth hormones in the environment, which reflected the direct or indirect risks for all living organisms (El-Shahaby *et al*. 2003).

The present investigation aimed to study the cytogenetic effects of naphthalene acetic acid and benzylaminopurine in onion root meristematic cells of onion to report not only if these phytohormones have a clastogenicity effects through chromosomal abnormalities induced but also to ameliorate their toxic effects on living organisms.

MATERIALS AND METHODS

Genetic materials

Onion bulbs were used in this study as a genetic testing material because it is easy to obtain root meristem cells and have a small number of chromosomes (2n = 16). Therefore, the commerciall onion bulbs (*Allium cepa* L., Family Amaryllidaceae) were randomlly collected from the local market in Mansoura city, Dakahlia Governorate and then subjected to sun-dried for two weeks. Then, the healthy dry bulbs were used for testing the cytogenetic effects of plant growth hormones.

Fixative solution

Due to the Carnoys solution (farmer's solution) which is the most common fixative solution. It consists of one part glacial acetic acid and three parts of absolute ethanol (Mirzaghaderi 2010) was used inthis study.

Acetocarmine staining

Carmine is a basic stain that was prepared from insect *Coccus cacti*. Acetocarmine stain was prepared by dissolve 10 g carmine in one litre of 45% glacial acetic acid, then boileezers and cooled for 24h. Filter into dark bottles and store at 4C°. In addition 10% ferric chloride solution was added to 100 ml of acetocarmine solution (Mirzaghaderi 2010) .

Hormones

1-Naphthaleneacetic acid (NAA) is an organic compound, its chemical formula C10H7CH2CO2H. This colorless solid is soluble in organic solvents. It consists of a carboxylmethyl group (CH2CO2H) linked to the "1-position" of naphthalene. It was a synthetic plant hormone from the auxin family used for the vegetative propagation of plants from stem and leaf cuttings via rooting horticulture products (Morikawa and Takahashi 2004). This hormone was toxic to plants at high concentrations. In the United States, products containing NAA require registration with the Environmental Protection Agency as pesticides.

6-Benzylaminopurine (BAP), it's chemical formula C12H11N5. It was a first-generation of synthetic cytokinin that elicits plant growth and development responses, setting blossoms and stimulating fruit richness by stimulating cell division (Teixeira da Silva and Jaime 2019). It is an inhibitor of respiratory kinase in plants, which increases post-harvest life of green vegetables. It's influence of on postharvest green color retention on broccoli heads and asparagus spears, showed positive results for quality retention. Treatment with 10 and 15 ppm BAP can be used to extend shelf life of fresh-cut broccoli florets and shredded cabbage during storage at 6±1°C of commercial level (Siddiqui *et al*. 2011).

Chromosome squash technique

Onion (*Allium cepa* L.) roots were used for cytogenetic bioassay. Root meristem raised in water was exposed to different concentrations of plant growth hormones. Root tips excised from treated and controlled materials were fixed in 1: 3 acidic alcohols for 24 hr and preserved in 70% ethyl alcohol

Transfer fixed root meristems to 1% acetocarmine and kept for one to two hours. Heat until the acetocarmine begin to boil, cut off the root cap with a razor blade and squeeze the meristematic tissue with a lancet needle in one drop of acetocarmine. Then add a caver slip over the squash meristematic tissue. (Sharma and Sharma 1980).

Remove and dry the increased stain of acetocarmine from the slide using a filter paper. Heat the slide to a point just below boiling. Then, quickly squash the meristematic tissue again carefully with thamb or for finger between two layers of filter paper without move the caver slip at this point. Then the slide was examined under the microscope and recorded the data shown was (Kwiton.1997).

Cytological traits

Different phases of mitosis were counted and chromosomal abnormalities were recorded. Mitotic index, phase indices and total abnormality percentage were calculated at different phases. Slides were tested and counts for dividing cells, non-dividing cells, cells at each mitotic phase and aberrant cells. Photomicrographs of some aberrant cells were recorded. Mitotic index was calculated as a number of dividing cells over the total number of cells counted expressed in percentage. The phase indices were estimated as a number of cells in each mitotic phase over the number of dividing cells expressed in percentage. Similarly, the percentage of abnormal cells was calculated as the number of aberrant cells over the number of dividing cells. (Lavania 1996).

RESULTS AND DISCUSSION

Germination of onion

Naphthalene acetic acid as shown in Figure 1 plays an important role in regulation the development of onion roots at 5 and 15 ppm. These concentrations increased the diameter of roots in relation to the concentrations of 10 and 20 ppm. This may be due to the increase in cellulose production by the cell leading to increase the size of cell wall. This agreed with Onyemaobi *et al*. (2012),who decided that decrease in root length may be due to enhanced lignin production that tend to

solidify the cell wall as a consequence restricts root growth. This indicated that when onion roots were subjected to NAA, there was an increase in root size through its reorganizing ability of cell wall. As shown in Figure 2, the different concentrations of benzylaminopurine (BAP) enhanced the length and biomass of onion roots.

5 ppm NAA **10 ppm NAA**

15 ppm NAA **20 ppm NAA**

Figure 1. Development of onion roots exposed to different concentrations of NAA after 7 days.

5 ppm BAP **10 ppm BAP**

15 ppm BAP **20 ppm BAP**

Figure 2. Development of onion roots exposed to different concentrations of BAP after 7 days.

This indicated that this phytohormone plays an important role as a signal molecule in regulating mitotic activity leading to increasing the biomass of roots. The results showed that BAP improved the initiation of roots, root length and biomass of roots via stimulated cell division. Furthermore, the concentrations of 10 and 20 ppm of BAP increased the biomass of roots in relation to other concentrations. It was evident that BAP stimulated the development of onion roots and increased the number and the length of roots. This stimulation may have to play an important role in absorbing water from the deepest location in the soil leading to decrease irrigated water needed. The results obtained herein reflected that when onion roots were subjected to naphthalene acetic acid, the number and the length of roots were increased due to mitotic activity of the merestematic cells. This indicated that phytohormones plays a significant roles in the regulation and initiation of plant physiological process (Truta *et al*.2011). Thus, phytoregulators are widely used in agriculture sector for controlling the growth of dwarf plant cultures inclusively at genetic level. However, plant growth hormones not only positively influence numerous aspects of plant growth, development and physiology, but also induced cytogenetic abnormalities (Kallak and Vapper 1985). This agreed with Mansour and Kamel (2005), who found that gibberellic acid stimulated mitotic activity and biochemical process in the cell.

Cyto-morphological abnormalities

Plant growth hormones are substances that have fundamental role in the regulation of plants life cycle. Cytokinins regulate plant growth through the exogenous application to some organs that normally lack these hormones via stimulated cell division. It was affect not only cell division but also many other physiological aspects as seed germination, shoot initiation and growth (Riou–Khamlichi *et al*. 1999). The results registered in Table 1 showed that the different concentrations of plant growth hormones induced morphological alterations in cell shape differed from one concentration to another. In addition, binucleate cells, ghost cells, pyknosis, necrosis, apoptosis and extended cells were obtained with different percentage at different concentrations. Meanwhile, the total number of aberrant cells were increased above the control values due to hormonal treatments. The percentage of aberrant cells varied greatly from one concentration to another with a dose-dependent. The concentration of 10 and 20 ppm of NAA was remarkably increased the percentage of aberrant cells, while the concentrations of 15 and 20 ppm BAP showed the same trend.

Table 1. Different types of cyto-morphological abnormalities induced in onion root tips exposed to different hormonal concentrations.

Hormone	Doses (ppm)	Analyzed cells	Morphological alterations in cell shape		Binucleate cells	
			Number of cells	%	Number of cells	%
Control	0	1945	27.0	1.38	2.00	0.10
NAA	5	2430	80.0	3.29	25.0	1.03
	10	3450	36.0	1.04	23.0	0.67
	15	2559	0.00	0.00	1.00	0.04
	20	1381	9.00	0.65	4.00	0.29
BAP	5	1831	1.00	0.06	42.0	2.29
	10	4635	7.00	0.15	104	2.24
	15	2965	49.0	1.65	13.0	0.44
	20	2175	9.00	0.41	5.00	0.23

Table 1. Continued.

Hormone	Doses (ppm)	Ghost cells		Nucleus loses(Pyknosis)		Necrosis cells	
		Number of cells	%	Number of cells	%	Number of cells	%
Control	0	0.00	0.00	15.0	0.77	0.00	0.00
NAA	5	0.00	0.00	10.0	0.41	0.00	0.00
	10	295	8.55	59.0	1.71	136	3.94
	15	145	5.67	125	4.88	2.00	0.08
	20	45.0	3.26	267	19.33	0.00	0.00
BAP	5	36.0	1.99	42.0	2.32	5.00	0.28
	10	2.00	0.04	210	4.53	6.00	0.13
	15	51.0	1.72	159	5.36	10.0	0.34
	20	0.00	0.00	62.0	2.85	3.00	0.14

Table 1. Continued.

Hormone	Doses (PPm)	Apoptosis		Extended cells		Total of aberrant cells	
		Number of cells	%	Number of cells	%	Number of cells	%
Control	0	4.00	0.20	22.0	1.13	70.0	3.60
NAA	5	11.0	0.45	187	7.70	315	12.46
	10	0.00	0.00	129	3.74	678	14.65
	15	0.00	0.00	78.0	3.05	351	13.72
	20	0.00	0.00	63.0	4.56	388	28.09
BAP	5	4.00	0.22	55.0	3.03	185	10.10
	10	0.00	0.00	192	4.14	521	11.24
	15	0.00	0.00	442	14.91	724	24.42
	20	4.00	0.18	430	19.77	513	23.58

Notes : NAA,Naphthalene acetic acid; BAP, Benzylaminopurine.

This indicated that the highest percentage of abnormalities was recorded in roots treated with 10 and 20 ppm of NAA, as well as, 15 and 20 ppm of BAP. Increased percentages of divided cells affected by hormones showed

more values of abnormalities if compared with a control value. These results agreed with Reyes *et al.* (1991), who reported that benzyl adenine stimulates DNA synthesis and also nuclear DNA polymerase activity during germination. Plant growth hormones accelerated the transport of protein into nuclei and thus shortened the time needed to the completion the cell cycle. Increasing the rate of aberrant cells affected by stimulators may be due to increasing the initiation of cell division cycle at earlier times than in control. This reflected that plant growth hormones stimulated DNA polymerase activity which is essential for DNA replication during the cell cycle. Increasing the percentage of aberrant cells above the control reflected that phyto-regulators enhanced mitotic activity above the control as reported before by Huyluoglu *et al.* (2008). These results indicated that the promotion of cell division may be due to increasing the rate of DNA synthesis (Reyes *et al.*1991) or via increasing the rate of cell division. Meanwhile, the morphological alternations of the cells reflected that plant growth regulators may affect on the length of the G_1 phase (Fujii *et al.*1999). In addition, increasing the number of extended cells under the effect of plant growth hormones may be due to longer period of division in the G_1 phase of the interphase or to high rapid of cell division or by combinations of these two phenomena (Wismer *et al.*1995). The results obtained herein indicated that phytoregulators used in this study do not have an inhibitory effect on protein biosynthesis used in spindle formation which leading to easy of chromosome splitting from kinetochores for promoting their separation and movement toward the opposite poles of the cell and consequently increased the number of cells in which the chromosomes reached the opposite poles .

Increased the percentages of abnormality cells above the control constitute a significant portion of genetic damage produced by plant growth hormones as shown before by Kaymak (2005). All the concentrations applied in this study were capable to cause a wide range of chromosomal abnormalities which generally ranged from 12.96 % to 28.09 % with NAA and from 10.10 % to 24.42 % with BAP. Similar results of chromosomal abnormalities have been earlier reported by many researchers tested the genotoxic effects of benzyl adenine on root tip cells of *Allium cepa* as Tabur and Demir (2010). The variation in the percentages of aberrant cells shown in this study was a dose dependent (Soh and Yang 1993). Nucleus loses, necrosis and apoptosis reflected the toxicity of plant growth hormones which probably led to cell death (Fiskesjö 1985). Loses of the nucleus may be resulted from vacuolization of the nucleus leading to conspicuous in the nucleoplasm in the root tip cells following the treatment with plant growth hormones. The percentage of cells loses nucleus was ranged from 0.41 to 19.33 with NAA and from 2.32 to 5.36 with BAP. Vacuolated nucleus was the major abnormality evident resulted from stimulators. This agreed with El-Ghamery *et al.* (2003),who found that the chromatin lost its stain ability or appeared as dense granulated and nuclei become vacuolated. Higher concentrations of NAA induced a variety of abnormalities in the meristematic cells. The action of plant growth hormones may be affected on the degree and on the rate of coiling (Guttman1956). The results obtained herein agreed with Firket *et al.*(1955), who found that 95 percent of all nuclei contained granular and filamentous material or were prophasic in appearance. In addition, Guttman (1956) reported that removal of RNA by ribonuclease does not prevent synthesis of DNA before prophase but inhibits the progress of mitosis. However, Sarma *et al.* (2017) reported that naphthalene is not mutagenic but does produce toxicity dependent secondary damage to DNA at excessive concentrations. Evidence for the secondary damage as DNA fragmentation, sister chromatid exchange and chromosome breakage, were consistent with a threshold – related cytotoxicity of tumour formation, driven by cytotoxicity and cell regeneration which leading to tumour formation.

Plant systems are sensitive biomonitors of the cytotoxic and genotoxic effects of different chemicals (Grant 1999). *Allium* assay test reflect the presence of cytotoxic or genotoxic effects of some chemicals. This assay can be used to protective from the effects of some chemical compounds. Application of exogenous cytokinin to some organs had been shown to induce cell division (Riou–Khamlichi *et al.*1999). From the results obtained by Mahfous *et al.* (2014), it is clear that both salicylic and jasmonic acids proved to induce a number of chromosomal aberrations in all mitotic phases. The yield percentage of aberrant cells implies higher cytological aberrations by BAP than NAA. Stimulator chemicals plays an important role in plant growth and development by regulating and stimulating cell division (Carle *et al.*1998) and triggering cell elongation (Howell *et al.*2007). Recently, much interest focused on the cytogenetic activity of plant growth regulators (Tabur and Demir 2009). The results obtained in this study are in accordance with Tabur and Öney (2012), who found that many of the stimulators produced abnormalities in chromosome structure and behaviors. It can be recommended that no need to add exogenously any stimulators without the stress condition was present. As known, some of plant growth regulators increased cell distortion and chromosomal aberrations even when stress conditions were not present (Tabur and Demir 2010). In addition, some aberrations may also resulted from stimulators. Furthermore, Carle *et al.* (1998) decided that cytokinins play an important roles in plant growth and development by promoting cytokinesis , regulating mitosis and increasing mitotic activity. However, gibberellins stimulate cell division, cell elongation and mitotic activity.

The ghost cells produced ranged from 3.26 to 8.55 % and 0.00 to 1.99 % in response to the treatments with NAA and BAP, respectively. This was a dead cell from which the outline was visible but nucleus and cytoplasmic structure was not stainability. Highest percentage of apoptosis cells was obtained from 5 ppm of NAA and 20 ppm of BAP. Apoptosis is a biological process an energy dependent which is genetically controlled by damaged cells. On other hand, the percentage of pyknosis was ranged between 0.41 – 19.33 % and 2.32 – 5.36 % by NAA and BAP, respectively. It is the irreversible condensation of chromatin in the nucleus of a cell undergoing necrosis or apoptosis. It is followed by karyorrhexis or fragmentation of the nucleus. The highest percentage of necrosis reached to 3.94 % of cells by NAA and 0.34 % of cells by BAP. It is a form of cell injury resulted from the premature death of cells in living tissue by autolysis. Necrosis caused by the unregulated digestion of cell components. In contrast,

apoptosis is naturally occurring programmed and targeted cause of cell die. While apoptosis often providing beneficial effects to the organism, necrosis providing detrimental fatal.

Abnormal chromosomal behavior

Mitotic irregularities resulted from the treatment with plant growth hormones were shown in Table 2. Chromosomal aberrations produced included anaphase bridges, chromosomal breaks, lagging chromosomes, stickiness chromosomes, c-mitosis, diagonal chromosomes.

Table 2. Abnormal chromosomal behaviour in meristemic root cells of onion exposed to different concentrations of plant growth hormones.

Hormone	Doses (ppm)	Analyzed cells	Ana- telophase bridges		Chromosomal breaks		Lagging chromosomes	
			Number of cells	%	Number of cells	%	Number of cells	%
Control	0	1945	1.00	0.05	0.00	0.00	0.00	0.00
NAA	5	2430	0.00	0.00	0.00	0.00	0.00	0.00
	10	3450	5.00	0.14	1.00	0.03	0.00	0.00
	15	2559	1.00	0.04	2.00	0.80	0.00	0.00
	20	1381	0.00	0.00	0.00	0.00	0.00	0.00
BAP	5	1831	5.00	0.28	2.00	0.11	1.00	0.06
	10	4635	2.00	0.04	3.00	0.06	0.00	0.00
	15	2965	4.00	0.13	3.00	0.10	1.00	0.03
	20	2175	3.00	0.14	1.00	0.05	0.00	0.00

Table 2. Continued.

Hormone	Doses (ppm)	Stickiness chromosomes		C- mitosis		Digonal cells	
		Number of cells	%	Number of cells	%	Number of cells	%
Control	0	5.00	0.26	0.00	0.00	0.00	0.00
NAA	5	7.00	0.29	1.00	0.04	0.00	0.00
	10	92.0	2.67	13.0	0.38	6.00	0.17
	15	7.00	0.27	2.00	0.08	3.00	0.12
	20	11.0	0.8	2.00	0.14	1.00	0.07
BAP	5	13.0	0.72	15.0	0.83	5.00	0.28
	10	47.0	1.01	9.00	0.19	12.0	0.26
	15	62.0	2.09	0.00	0.00	8.00	0.27
	20	7.00	0.32	0.00	0.00	13.0	0.60

Table 2. Continued.

Hormone	Doses (ppm)	Abnormal kinetics		Total of aberrant cells	
		Number of cells	%	Number of cells	%
Control	0	8.00	0.41	14.0	0.72
NAA	5	6.00	0.25	14.0	0.57
	10	0.00	0.00	117	3.39
	15	5.00	0.20	20.0	0.78
	20	0.00	0.00	14.0	1.01
BAP	5	18.0	0.99	59.0	3.22
	10	50.0	1.08	123	2.65
	15	23.0	0.78	101	3.41
	20	20.0	0.92	44.0	2.02

Notes: NAA, Naphthalene acetic acid; BAP, Benzylaminopurine.

Mitotic irregularities such as c-mitosis (Figure 3) may be result from spindle dysfunction. Lagging chromosomes may be resulted from chromosomes lost their movement abilities due to growth regulators. Therefore, they get stuck in anywhere and cannot go to final destination. These aberrations are accepted as indicator of toxicity which results in cell death. Anaphase bridges (Figure 4) may happen from dicentric chromosomes. In this study, chromosomal aberrations are remarkably increased in the treatments with plant growth hormones as compared to control.

The concentrations of 10 ppm of NAA and 15 ppm BAP produced the highest number of aberrant cells. However, abnormal kinetics was increased by BAP in relation to NAA. In addition, the concentration of 10 ppm from NAA produced the highest number of stickiness cell chromosomes (Figure 5). Meanwhile, the concentration of 15 ppm BAP appeared the same trend for stickiness chromosomes. The highest percentage of anaphase bridges and c-mitosis was resulted from 10 ppm NAA and 5 ppm BAP. The occurrence of mitotic bridges may be attributed either to chromosome stickiness or fusion between chromatids or by the loss of telomere capping or from dicentric chromosomes or by breakage probably formed and fusion of chromatids (Türkoglu 2007). The breaking chromosomes may be result from the depression of energy systems which interference with DNA synthesis in s-phase, protein synthesis and binding affected on the integrity of the chromosome leading to play a significant role in fragmentation (Umebese *et al.*2013). In this regard chromosomal aberrations (Figure 6) can be considered as indicators for clastogenicity.

Stickiness chromosomes may be resulted from immediate reactions with DNA protein cross linking (Amin 2002). This agreed with Odeigah *et al.* (1997), who reported that sticky chromosomes are indicators of high toxicity as irreversible physiological effect leading to cell death. The highest percentage of c-metaphase cells was reached to 0.38 % of cells at 10 ppm NAA and 0.83 % of cells at 5 ppm BAP. C-metaphase may be due to the complete inhibition of spindle formation by the action of hormones on the microtubules. This indicated that these hormones may caused complete inhibition of mitotic spindle fibers leading to irregular movement of

daughter chromosomes to the opposite poles at anaphase. C-metaphase indicates the action of hormones on the formation of spindle fibers involved in the processes of chromosome movement through regulation and control of de-polymerization and polymerization of the microtubules leading to suppress mitotic cycle (Soh and Yang 1993). As a consequence of this disorder, the cell cycle was interrupted in metaphase leading the chromosomes are seen scattered and condensed with very well defined centromeres (Fiskesjo 1985). The formation of disturbed metaphase may be due to the inhibition of the respiratory pathways resulted low energy production which necessary for the movement of chromosomes (Ajay and Sarbhoy 1987).

In this study, lagging chromosomes were observed in low percentage following the treatment with BAP, it was ranged between 0.00 - 0.06 of % cells, while the treatment with NAA did not shown any lagging chromosomes. The induction of lagging chromosomes may be attributed to the failure of spindle apparatus or to the pro-metaphase movement accompanied by the adhesion of the centromeres of one or more chromosomes to the inner surface of plasma membrane and movement the others toward the equatorial plate led to the appearance of lagging chromosomes (Turkoglu 2007). This agreed with Gari *et al*. (1998), who decided that laggards are the direct results of breaks and fragmentation leading to the loss of centromeres and stopping their movement. In this respect, Fiskesjo (1985) described laggards chromosomes as a weak c-mitotic effects indicating risk of aneuploidy. Consequently, it was leading to the separation of unequal number of chromosomes in the daughter nuclei and then formed unequal cells sized or irregularly shaped nuclei. These results were also agreed with Fiskesjo (1985), who reported that laggards may be resulted from a weak c-mitotic effects which leading to a risk of aneuploidy.

Diagonal cell (depolarized ana-telophase) (Figure 7) was another type of abnormalities observed in this study which percentage ranged between 0.0 – 0.17 % and 0.26 – 0.60 % cells per analyzed cells pretreated with NAA and BAP, respectively. In this type of abnormalities, the two anaphase groups of chromosomes did not orient at the same axis of the cell. This may due to the indicative ability of plant growth hormones to interfere with spindle apparatus mechanism (El-Ghamery and Mousa 2017). This indicated the genetic risks of plant growth hormones in agricultural productivity. BAP treated cells scored the highest abnormal kinetics of chromosomes in mitotic cells which ranged between 0.78 – 1.08 % per analyzed cells. Abnormal chromosomal kinetics (Figure 8) is a common genetic phenomenon may be resulted from behavior changes induced by hormonal treatment (Reddy and Rao 1978).

The total percentage of aberrant cells was ranged between 0.57 – 3.39 % and 2.02 – 3.41 % for NAA and BAP treatments, respectively.

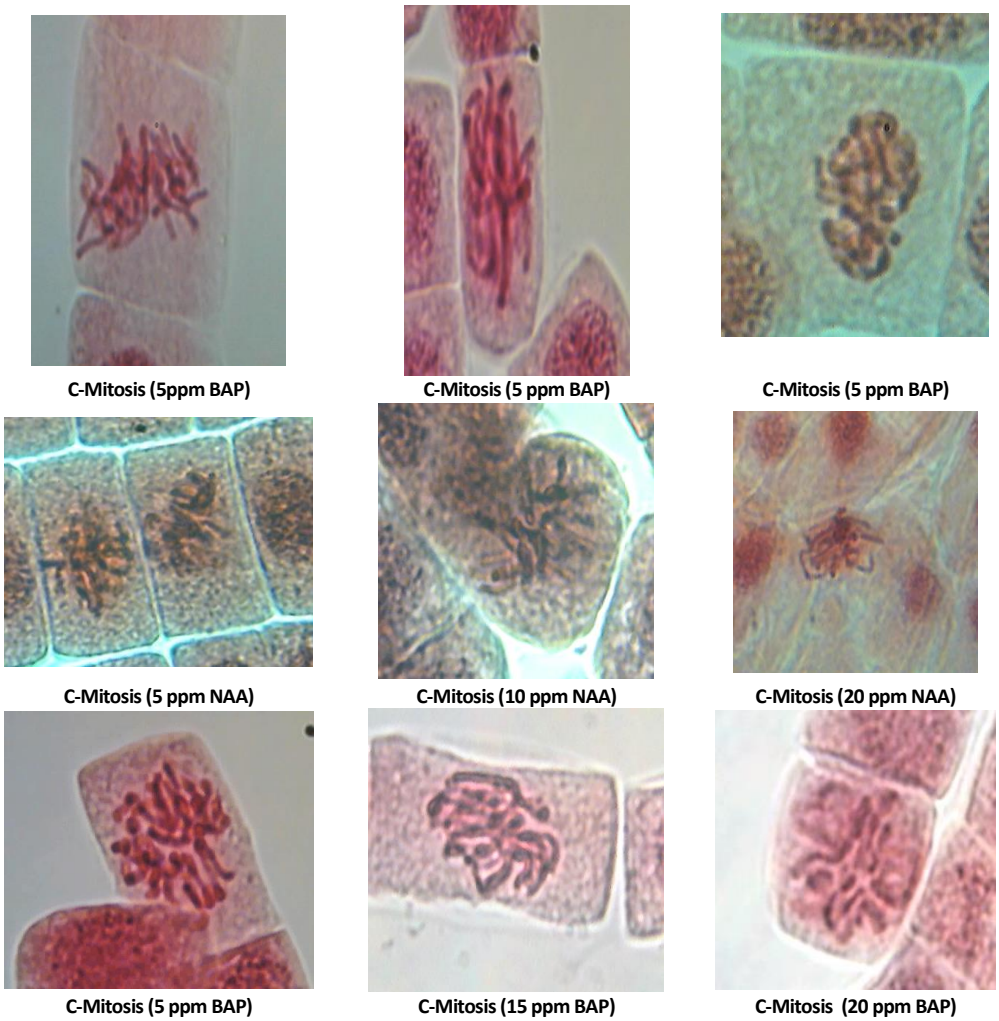
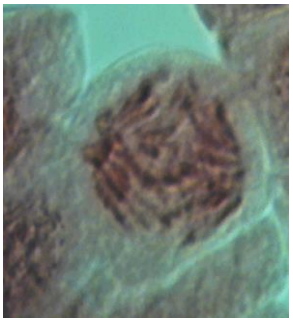
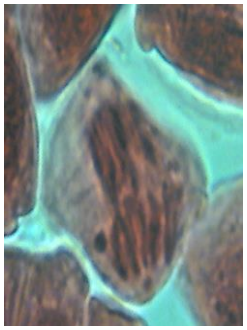


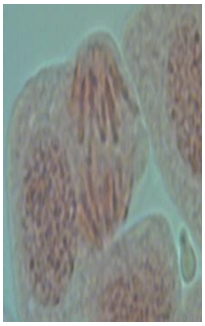
Figure 3. C- mitosis induced in onion root merestematic cells after pretreatments with phytohormones.



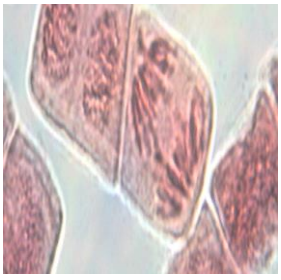
Bridges (10 ppm NAA)



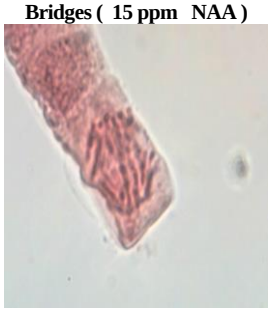
Bridges (15 ppm NAA)



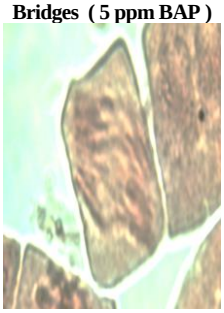
Bridges (5 ppm BAP)



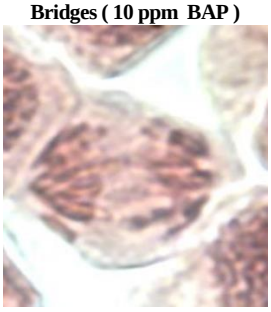
Bridges (10 ppm BAP)



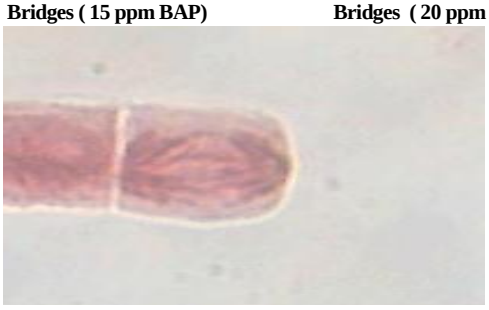
Bridges (15 ppm BAP)



Bridges (20 ppm BAP)



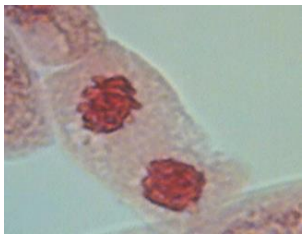
Bridges (20 ppm BAP)



Bridges (10 ppm BAP)

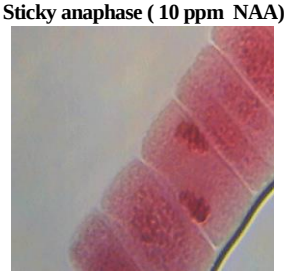
Figure 4. Mitotic bridges induced in onion root merestematic cells after pretreatments with phytohormones NAA and BAP.

Sticky anaphase (10 ppm NAA)

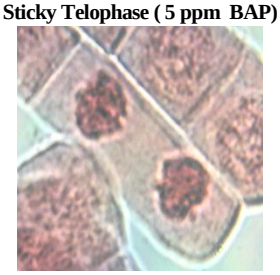


Sticky Telophase (5 ppm BAP)

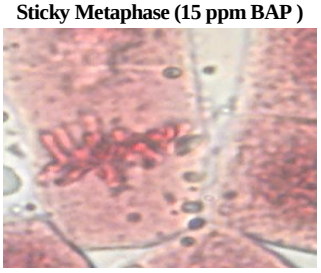
Sticky Metaphase (15 ppm BAP)



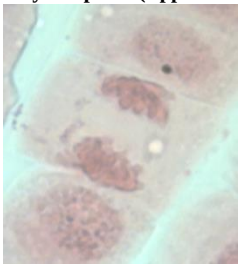
Sticky Anaphase (5 ppm BAP)



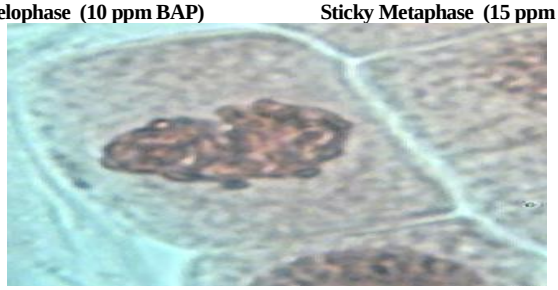
Sticky Telophase (10 ppm BAP)



Sticky Metaphase (15 ppm BAP)



Sticky Anaphase (15 ppm BAP)



Sticky Prophase (15 ppm BAP)

Figure 5. Sticky chromosomes induced in onion root merestematic cells after pretreatment with phytohormones NAA and BAP.

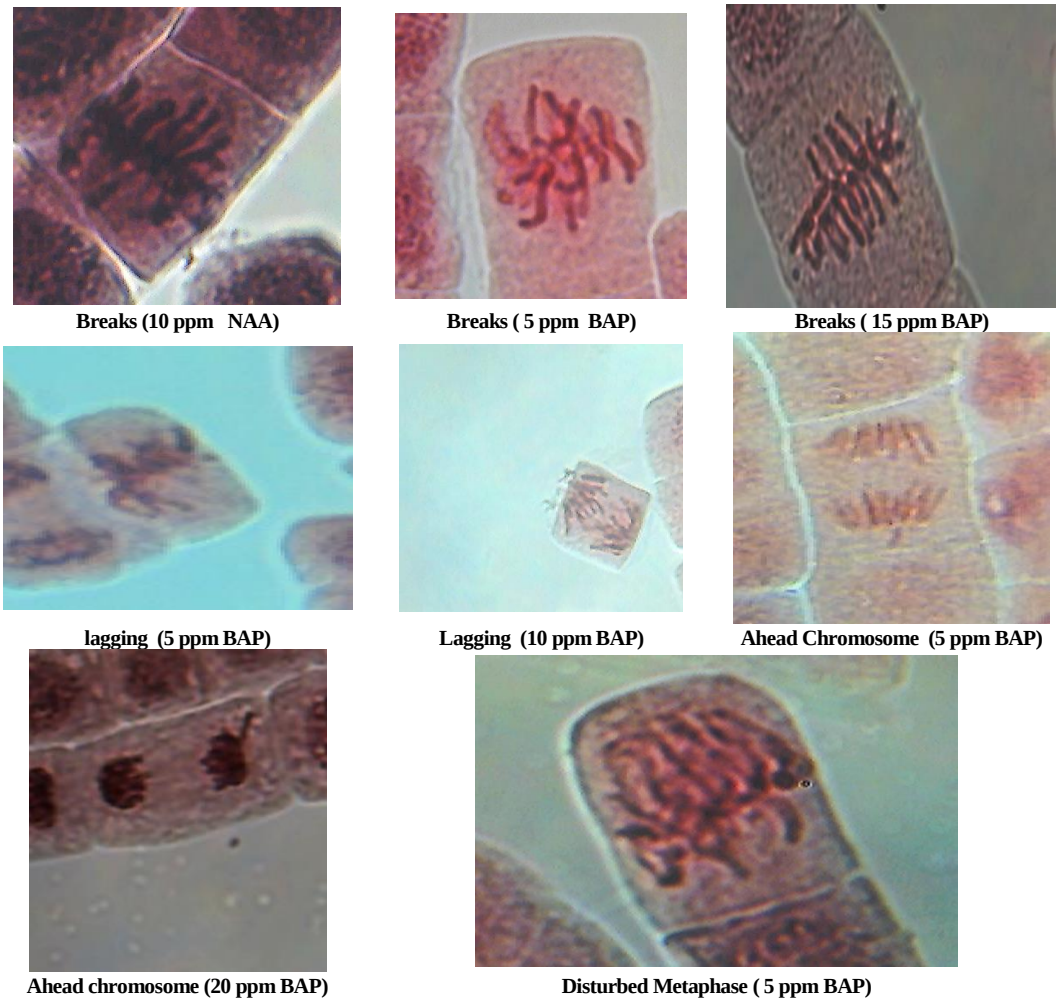


Figure 6. Chromosomal abnormalities induced in onion root merestematic cells after pretreatments with phytohormones NAA and BAP.

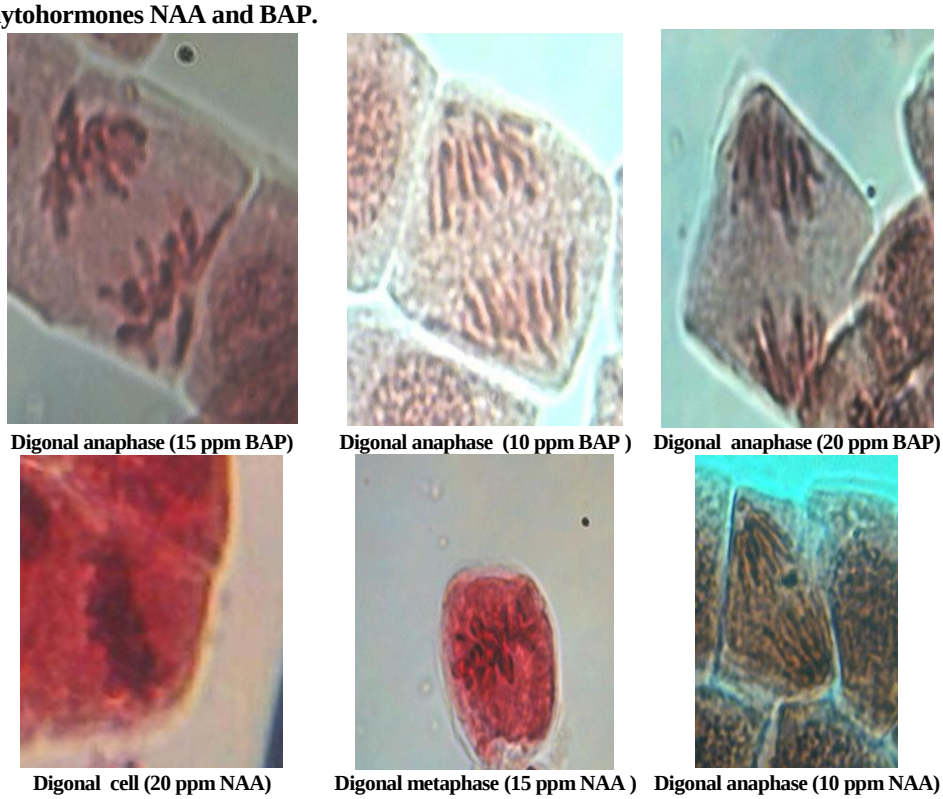


Figure 7. Digonal anaphase and metaphase in onion root merestematic cells after pretreatments with phytohormones NAA and BAP.

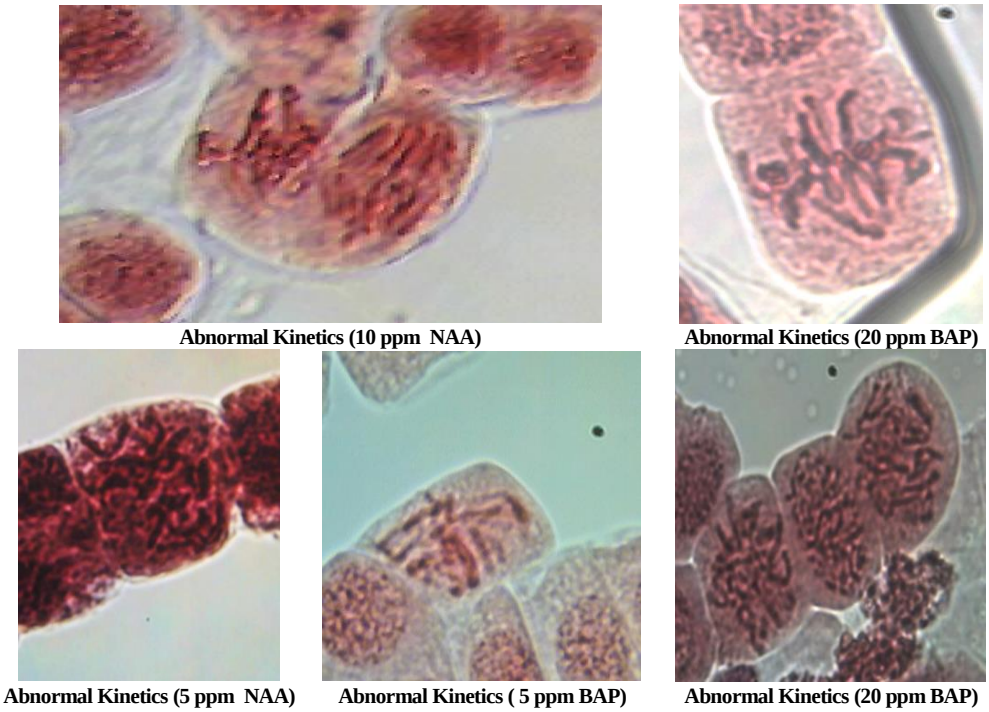


Figure 8. Abnormal kinetics of chromosomes in mitosis of onion root merestematic cells after pretreatments with phytohormones NAA and BAP.

This indicated the aberrant behavior of chromosomes in plant cells exposed to plant growth hormones. This indicated that hormonal treatments caused various karyological irregularities in mitotic cells *in vivo*. BAP showed a higher number of mitotic abnormalities than NAA. The observed changes indicated that most of these abnormalities may be due to complete or partial inhibition of spindle formation by the action of phyto-stimulators on the microtubules. Furthermore, these effects cased irregular movement of daughter chromosomes to the opposite poles at anaphase. Support of this view is the results obtained by Gul *et al*. (2006) about c-metaphase produced by hormonal herbicide avenoxan which produced multipolar anaphase. Similar observations were also obtained by the plant hormone homobrassinolide which produced a number of mitotic abnormalities in barley roots (Kartal *et al*.2009).

As shown before by Partanen (1963), meristematic locations in shoot and root tips naturally undergo diploid chromosomes, unlike non-meristemtic locations, which may contained different chromosome numbers resulted from somatic chromosome inconsistency. Different factors may be involved to cause this incidence, as abnormal spindle that disturbs cell multiplication, chromosomal breaks and continued DNA synthesis without mitosis. These affects on chromosomal content in the cells which may be augmented or significantly reduced (Partanen 1963). Furthermore, abnormal development of spindle fibers affected on the separation of chromosomes (Sunderland 1973) leading to laggard chromosomes as shown in this study. This concomitantly increases the DNA content in the cell. Phytohormones involving in the induction of $CycD_3$ expression encoding the D-type cyclin, which played a significant role in transition from G_1 to M phase during cell cycle (D`Agostino and Kiebe .1999) According to Sho and Yang (1993), kinetin increased mitotic activity and caused mitotic abnormalities in onion. The action of stimulators in mitotic interference as the toxic chemicals often termed as "mitotic poison". This may be due to their acute interference and abnormality of spindle apparatus formation, caused c-metaphase effect or laggards chromosomes (Ateeq *et al*.2002). In addition, c-mitosis yielding lagging chromosomes and cell multipolarity (Haliem 1995).

Mitotic index and incidence of mitotic phases

Mitotic index was considered as an indicator of cell proliferation biomarker. Though, the cytotoxic level of hormonal substances can be evaluated based on the increase or decrease in mitotic index. The significance reduction in mitotic index seen by hormonal treatments may be due to the inhibition of DNA synthesis or to the blocking G_2 phase of cell cycle. The cytotoxic effects of hormonal substances were estimated on the basis of changes in mitotic index and other chromosomal abnormalities. Thus, mitotic index reflects the frequency of cell division and the rate of seedling growth. As shown from the results registered in Table (3) the hormonal substance NAA drastically decreased mitotic index (MI) below the control group which ranged between 2.32 – 4.57 %. Meanwhile, BAP showed a dose – response for gradually decreasing mitotic index which ranged between 12.19- 6.62 % . Mitotic index is not only a parameter of intensity cell division, but also as indicator of cytotoxicity. This agreed with Marcano *et al*. (2004), who reported that cytotoxicity was defined as a decrease in mitotic index and as the increase in cell fraction including c- mitosis, multipolar anaphase, sticky chromosomes and laggards. The decline in mitotic index could be due to the inhibition of DNA synthesis (Truta *et al*. 2011). The inhibition of cell cycle proteins was a possible target site of hormones . In addition, the inhibition of DNA – polymerase necessary for the replication of DNA precursors, as well as other enzymes involved in cell cycle are more directly related with spindle assembly or orientation, all of these could explain the mitodepressive effect as reported before by Hidalgo *et al*.(1989).

Table 3. Mitotic index of meristemic root cells in onion treated with different concentrations of plant growth hormones.

Hormone	Doses (ppm)	Analyzed cells	Number of dividing cell	Mitotic index %	Phase index			
					Prophase cells		Metaphase cells	
					Number of cells	%	Number of cells	%
Control	0	1945	169	8.65	10	65.09	12	7.10
NAA	5	2430	54.0	2.22	21	38.89	7	12.96
	10	3450	146	4.23	49	33.56	44	30.14
	15	2559	117	4.57	82	70.09	10	8.55
	20	1381	32.0	2.32	10	31.25	11	34.38
BAP	5	1831	221	12.19	99	44.80	42	19.00
	10	4635	440	9.49	224	50.91	83	18.86
	15	2965	262	8.84	88	33.59	81	30.92
	20	2175	144	6.62	52	36.11	37	25.69

Table 3. Continued.

Hormone	Doses (ppm)	Phase index			
		Anaphase cells		Telophase cells	
		Number of cells	%	Number of cells	%
Control	0	12	7.10	35	20.71
NAA	5	10	18.52	16	29.63
	10	38	26.03	15	10.27
	15	15	12.83	10	8.55
	20	8	25.0	3	9.38
BAP	5	23	10.41	57	25.79
	10	50	11.36	83	18.86
	15	39	14.89	54	20.61
	20	22	15.28	33	22.92

Notes : NAA; Naphthalene acetic acid; BAP; Benzylaminopurine.

The results obtained in this study showed decline the mitotic index below the control involved the treatments with NAA. In contrast, the most concentrations of BAP increased mitotic index above the control group. These results indicated cytotoxicity of hormones used in this study. Concerning the frequency of mitotic phases, the decreasing order generally was the following, prophase > telophase cells. Meanwhile, at most concentrations metaphases > anaphase cells. Prophase cells were declined than the control group, while metaphase and anaphase cells were increased above the control. This indicated that plant growth stimulators may affect on chromosomes coiling in prophase and also can produce abnormalities in chromosome structure and behaviors. This are in harmony with Tabur and oney(2012), who reported that decline in prophase cells may be due to the uncoiled chromosomes affected by growth regulators leading to the disorderly chromosome contractions phyto-stimulators used in this study were successful in producing varying degrees of phase index. Alleviating the detrimental effect of stimulators on chromosomal behavior in mitosis leading to the frequency of mitotic abnormalities. This study explained that mitotic activity explained the effect of hormonal treatments leading to irregular mitosis. This agreed with Demir (2010), who found that some stimulators increased the cell distortion and chromosomal aberrations. In this respect, Rieder and Salmon (1998) reported that accurate chromosome segregation requires that sister kinetochores attach to microtubules emanating from opposite spindle poles which is a stochastic process, its error prone can result in chromosome malorientation. Mitotic irregularities may be mainly resulted from these reasons or spindle dysfunction. Adherent chromosomes may be resulted from sub-chromatid linkage between chromosomes or chromosomes lost their movement ability due to the effect of growth regulators. This explained the physical adhesion of chromosomal proteins (Patil and Bhat 1992). The division of cells and progression through the successive phases of cell cycle were affected by stimulators which increased the phase index of metaphase and anaphase above the control group, while prophase index was declined below the control. This was driven by a common class of protein kinases whose activity depends on the association with different classes of cyclins (Binarove *et al*.1998). The decline in phase index below the control may be due to some blocks happens in the cell cycle progression phases after treatments with hormones which may resulted from the suppression of cycle dependent kinases (CDKs). On the other hand, the inhibition in mitotic phases progress may be attributed from the increase in mitotic abnormalities and DNA damage (Enan 2009). From the data obtained in this study, it is clear that plant growth stimulators proved to induce a number of chromosomal abnormalities in all mitotic phases which may be due to their action on chromosomal proteins and DNA synthesis.

Therefore, more attention must be taken to evaluate the mutagenicity of stimulators on the genetic level of human using a number of mutagenicity tests. Stimulators acts as potent spindle inhibitor due to chromosomes lie on the metaphase plate instead of moving towards the opposite poles of the cells leading to induce c-metaphase. Therefore, it is important to determine whether these stimulators are safe for animal and human health. Application of hormones induced spindle disturbances associated with defective movement of chromosomes and chromosomes stickiness leading to chromosome bridges at anaphase or chromosome clumping at metaphase. In conclusion, the results obtained in this study justify detailed information about the genetic risks of phyto-regulators used herein because of stronger cytogenetic effects induced in onion root tips. They decrease

mitotic index attributed to blocking mitotic cycle and accumulation the cells in G_1/S or G_2/M transition point. Exposure to plant growth hormones cause abnormal chromosomal behavior in mitosis as c-metaphase, binucleate and tripolar cells, stickiness chromosomes, ghost cells, diagonal anaphase and metaphase, laggards chromosomes etc... They are caused binucleate cells resulted from a cytokinetic mitosis. Consequently, it is thought that convenient growth regulators might stop in crop production because of their adverse effects on chromosomal structures and behavior in mitosis. This study hypothesized that stimulators may affect morphological changes not only through the activity of cell division but also the cell cycle.

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التاثيرات السيتولوجية للنفثالين أستيك أسد والبنزايل أمينو بيورين في خلايا القمم النامية لجذور نبات البصل .

ميرفت إبراهيم كمال ، خليفه عبد المقصود زايد ، مصطفي خليل حسين و أشرف حسين عبد الهادى
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تهدف هذه الدراسة إلي فحص التأثيرات السيتولوجية المستحدثة بواسطة إثنين من هرمونات النمو النباتية هما النفثالين أستيك أسد (NAA)، البنزايل أمينوبيورين (BAP) في خلايا القمم النامية لجذور البصل . تم إجراء تحليل مقارن بين دليل الإنقسام الميتوزي ، وأنواع الخلل الكروموسومي الحادث في المرحلة الإستوائية بعد إنبات البصل في تركيزات مختلفة من كل هرمون من هرمونات النمو المستخدمة. ونتج عن كل من هرموني النمو زيادة في معدل الإنقسام الخلوي هذا بالإضافة إلي الخلل الحادث في تكوين خيوط المغزل والذي نتج عنه خلل في حركة الكروموسومات المتماثلة إلي الأقطاب المتضادة للخلية . تم الحصول على أعلي نسبة من التغيرات في شكل الخلية بواسطة تركيز 5 جزء في المليون من حامض النفثالين أستيك ، بينما أظهرت المعاملة بالبنزايل أمينوبيورين إستجابة للجرعة بالنسبة لزيادة نسبة الخلايا المطاطية . نتج عن كل المعاملات بهرمونات النمو المستخدمة زيادة نسبة التغيرات الكروموسومية عن تجربة المقارنة. كانت معظم التغيرات الظاهرية السائدة تشمل الخلايا متعددة الأنوية ، الموت الخلوي ، الإختفاء النووي ، الخلايا المطاطة ، necrosis. تعكس هذه التغيرات سمية هرمونات النمو علي الخلايا مما يؤدي إلي موت الخلايا المتأثرة بها. تشتمل معظم التغيرات السيتولوجية السائدة المرحلة الإستوائية التي تتسم بالبعثرة الكروموسومية ، القناطر الكروموسومية ، الكسور الكروموسومية ، لزوجة الكروموسومات ، digonal cells والتي تعكس التاثير الوراثي السام لهذه الهرمونات . ظهور هذه التغيرات الكروموسومية غير المعتادة في الخلايا المنقسمة يعكس تأثير هرمونات النمو علي تكوين خيوط المغزل مما نتج عنه البعثرة الكروموسومية ، التلكؤ الكروموسومي ، تعدد الأقطاب الخلوية . أدت كل المعاملات بهرمونات النمو المستخدمة إلي تغيير نسبة دليل الإنقسام الميتوزي والتي إنخفضت نتيجة المعاملة بحامض النفثالين أسيتك أو إرتفعت نتيجة المعاملة بالبنزايل أمينوبيورين مقارنة بتجربة الكنترول . لذا يمكن أن نستنتج من هذه الدراسة أن هرمونات النمو لها تأثير سام علي الخلايا مميزة النواة من خلال التغيرات الكروموسومية التي أظهرتها في هذه الدراسة .