Anti-mutagenic Effect of some Nutrients against the Mutagenecity of Colchicine after Induced Tetraploid Plants of *Nicotiana alata*

Elham A. A. Abd El-Hady[1*], Nagwa R. Abd El-Hamid[1], Atef A. A.Haiba[1], El-Morsy Sh. I.[2] and Ahmed Y. Mohamed[2]

[1]Department of Genetics and Cytology, National Research Center, Dokki, Giza, Egypt [2]Department of Botany, Al-Azhar University, Cairo, Egypt.
[*]elhamabdelhady@hotmail.com

Abstract: The present study aimed to induce tetraploid plants of *Nicotiana alata* by treating seeds in an aqueous solution of colchicine and identify the ploidy level by counting the chromosomes number. After that, the mutagenic potentialities of colchicine on the meiotic cells of tetraploid plants were determined and investigate the repair effects of the three applied nutrients (N, P and K) against colchicine at cytological level. Also examine the pollen grains viability at pre and post treatments with the nutrients. Cytological analysis revealed highly significant increase in the frequencies of chromosomal abnormalities induced by different concentrations of colchicine in which the percentage of abnormalities increased as the colchicine concentrations increased. Different types of chromosomal abnormalities were observed, such as stickiness, bridges laggards, micronucleus and ring chromosome. Nutrient treatments of tetraploid plants resulted in a remarkable reduction in the percentages of chromosomal abnormalities and revealed their repair effect against mutagenicity of colchicine at cytological level. The frequency of non viable pollen grains was increased as the concentration of colchicine increased but its percentages were reduced after treatment the tetraploid plants with the applied nutrients. It was concluded that, chromosome doubling has been used to obtain new ornamental characteristics of *Nicotiana alata* plants and the applied nutrients act against mutagenicity of colchicine and increase the pollen fertility in colchitetraploid plants. [Journal of American Science 2010; 6(9):860-869]. (ISSN: 1545-1003).

Key words: *Nicotiana alata*, Colchicine, Chromosomal abnormalities, Nutrients

1. Introduction:

The Family Solanaceae is found throughout the world but is most abundant and widely distributed in the tropical regions and very few members are found in temperate regions. The genus Nicotiana consists of 64 recognized species, some species are cultivated as drug or ornamental plants (Raju *et al*., 2009). *Nicotiana alata* species 2n = 18 chromosome, is called flowering tobacco. It is mainly grown as ornamental plant.

During the past fifty years, the development of the colchicine treatment as a means of producing polyploids has resulted in an increased interest in autotetraploid fodder cultivars. One of the major breeding problems was increase the seed fertility in autotetraploid plants. So, several attempts have been made to investigate the relationship between the seed fertility and the regularity of the meiosis in different crop species (Moore, 1963).

Colchicine is considered as a point mutagen at the molecular level and has a powerful effect on changing allele frequencies (Castro *et al*., 2003). Among the conventional methods for evaluating genetic damage of some chemicals are chromosomal abnormalities for assessing the genotoxicity induced by these chemicals. Singh (1992) noticed that induced autotetraploid of *Petunia hybrida* by treating with colchicine and found a large proportion of chromosomal abnormalities such as laggards, bridges and tripolar with decrease in pollen fertility. Colchicine with various concentrations was given to the apical buds of *Zea mays* L. plants to induce tetraploidy and revealed highly irregular meiosis in the C1 generation, these meiotic abnormalities increased pollen sterility which leads to lower seed setting (Hassan and Ahmed, 1999). Also, colchicine exhibited toxicity to plant cell by reducing anther culture response through plant generation from embryoids and survival of plantlets in *Nicotiana tabaccum* L. (Burun and Emiroglu, 2008).

Many investigators studied the repairing effects of some nutrients and revealed their repair effect against some chemicals such as fungicide (Ali, 1996), herbicide (El- Nahas, 2000), insecticide (Hassan, 2000 and Hassan *et al*., 2002). Also, (Shehata *et al*., 1999) noticed a reduction in the ability of colchicine as a mutagen to arrest mitosis when combined it with water extracts of some

medicinal plants, they interpreted the antimutagenic effects of such water extracts against colchicine due to their counteraction effect.

The present investigation aimed to study the mutagenic effects of colchicine on the meiotic cell division of tetraploid plants and investigate the antimutageneic effects of the applied nutrients against colchicine at cytological level.

2. Materials and Methods:

Seeds of *Nicotiana alata* were immersed for 24 hours in distilled water, then soaked in aerated colchicines solution with different concentration 0.10, 0.25 and 0.50 % (w/v) for 12, 24, and 48 hours before transfer to the pots and after growing, seeds of tetraploid plants were obtained from the plants treated with the highest concentration of colchicine (0.50%) and confirmed its ploidy level (4n) by counting the chromosomes number. The germinated seedling of either diploid or tetraploid plants were treated by N, P and K. Nitrogen was added as Urea [CO $(NH_2)_2$ 46% N] at rates of 0.0, 50, 100 and 150 kg/Fed. while phosphorus was added as ordinary super phosphate (15.5 % P_2O_5) at rates of 0.0,50, 100 and 150 kg/Fed. and potassium was added as potassium sulphate (K_2SO_4 48% K_2O) at rates of (0.0, 40, 80 and 120 kg/Fed).

At the flowering stage, six plants were selected from tetraploid plants induced by the three concentrations of colchicine (0.10, 0.25 and 0.50%) and control to collect flower buds for testing the mutagenicity of colchicine. Also, the flower buds were collected from the tetraploid plants which previously were induced by 0.50% colchicine, then treated separately with different concentrations of N, P and K nutrients after 12, 24 hours and 15 days from the last treatment of the three applied nutrients to evaluate its repairing effect against colchicine. Cytological analysis were carried out on the flower buds pre and post treatments with N, P and K nutrients using aceto-carmine smearing technique according to Belling (1920). Stainability of pollen grains of *Nicotiana alata* in aceto-carmine stain was used as an index for determining pollen grains viability after treatments with all concentrations of colchicine and the N, P and K nutrients according to Moreira and Gurgel (1941). All obtained cytological data from the different treatments were statistically analyzed using *t*-test.

3. Results and Discussion:

Mutagenecity of colchicine:

The results indicated that colchicine could be efficiently utilized for the induction of tetraploid plants of *Nicotiana alata* (4n) and confirmed by chromosome number which is the only reliable method to confirm the ploidy levels (Singh, 1993). Table (1) represents the potentiality of the colchicine to induce a high percentage of abnormal PMCs as compared with the control. After 48 hours treatment with the highest concentration of colchicine 0.50 % the maximum percentage of abnormal PMCs was reached at value of 19.03% as compared with the control value of 0.83%. Flower buds of the tetraploid plants showed higher frequencies of meiotic irregularities than the diploid ones where, colchicine induced a wide range of abnormalities and had highly significant effect to increase the frequencies of these abnormalities at all applied concentrations and different times of duration. This result was supported by Burun and Emiroglu, (2008) they concluded that colchicine treatments exhibited toxicity to plant cells. Also, Singh, (1992) induced autotetraploid of *Petunia hybrida* by treating with colchicine and found laggards, bridges and tripolar abnormalities with decrease in pollen fertility.

The treatment of *Nicotiana alata* with three concentrations of colchicine resulted in a marked increase in the percentage of abnormal PMCs in the 2nd meiotic division than in the 1st one in all treatments as recorded in Table (1). At the 1st division the maximum value of abnormal PMCs was 17.52 % after 48 hours with the highest concentration, while in the 2nd division the highest frequency of abnormal PMCs was 21.48 % after 48 hours treatment with the same concentration. The increase of abnormal PMCs in the second meiotic division were observed due to the high frequency of disturbed poles, bridges and multipolarity which were recorded in these stages and which lead to their accumulation in the 2nd meiotic division as mentioned by Mendes-Bonato *et al.* (2009). The same results obtained by Hassan (1991) who observed an increase in the percentage of aberrations in anaphase Π after treating *Vicia faba* flower buds with pesticides. Thus, it could be concluded that colchicine treatment affected the spindle function and delayed the division of the meiotic cycle.

The flower buds of the tetraploid plants showed different types of abnormalities covering all the meiotic stages with variable frequencies as shown in Fig.(1). Stickiness represented the most conspicuous type of abnormalities observed after all treatments. Chromosomal stickiness was found to cover the whole chromosome complement leading to the appearance of chromatin masses where the general appearance of the chromosomes was lost. It has been suggested by Patil and Bhat (1992) that stickiness is a type of physical adhesion involving mainly the proteinaceous matrix of chromatin

material. Such type of irregularity was also reported by Promilla and Bhattacharya (1991).

Another common type of abnormalities was the induction of disturbed configuration, disturbed pole position in anaphase II were observed in Figure (1). Disturbed and scattered chromosomes considered the most common type of meiotic abnormalities. It was produced with high frequencies at the highest concentration of colchicine. This type of abnormality was produced as a result of inhibition and disturbance of spindle as mentioned by Omran and Mohammed (2008), they concluded that, the colchicine is an anti-mitotic agent that can bind to microtubules and inhibit formation of spindle fibers during the cell division resulting in formation of the polyploid cells.

Table (1): Percentage of abnormal PMCs and type of abnormalities of *Nicotiana alata* plants after treatment with different concentrations of colchicine at 12, 24 and 48 hour.

Duration	Conc. gm/l	Mean % of total abnormal PMCs	% 1St M.D.	% 2nd M.D.	% types of abnormalities						
					sticky	Dist	laggard	bridge	Mult-polar	Micro-nu	Ring
12 hour	C	0.78±0.10	0.85	0.72	0.17	0.15	0.10	0.08	0.08	0.10	0.10
	0.1	6.85**±0.49	6.45	6.64	29.20	22.38	16.79	11.92	8.52	6.81	4.38
	0.25	7.33**±0.42	6.64	7.36	30	21.82	17.05	11.36	8.41	6.82	4.55
	0.50	8.18**±0.56	8.03	10.81	30.55	20.37	16.29	11.20	8.15	6.72	5.50
24 hour	C	0.87±0.10	0.98	0.73	0.18	0.17	0.13	0.10	0.08	0.12	0.08
	0.1	6.62**±0.38	6.65	6.73	28.97	19.14	13.35	13.10	11.84	6.30	5.04
	0.25	9.80**±0.21	9.39	10.10	30.61	20.07	16.84	12.76	9.69	5.10	4.93
	0.50	12.97**±0.46	15.93	10.30	30.21	19.28	16.07	13.11	10.41	5.66	5.27
48 hour	C	0.83±0.10	0.80	0.88	0.18	0.17	0.12	0.10	0.08	0.08	0.08
	0.1	10.03**±0.36	9.15	11.59	28.07	21.93	15.12	12.79	8.14	7.31	6.64
	0.25	13.83**±0.43	13.07	15.11	30.36	19.88	14.82	12.17	9.52	7.11	6.14
	0.50	19.03**±0.45	17.52	21.48	33.19	18.39	14.71	12.52	8.58	6.83	5.78

* Significant from control at 0.05 level (*t*- test) ** Significant from control at 0.01 level (*t*- test)

Among the most common type of meiotic abnormalities exerted by colchicine were PMCs with laggards. This type of abnormality was observed in both first and second meiotic division in all treatments. Figure (1) showed lagging bivalent at metaphaseI. The induction of laggards at metaphase I may be extending in all subsequent meiotic stages. In this respect many phases such as, anaphase I, telophase I, metaphase II and anaphase II with laggard were observed. The induction of laggards could be attributed to irregular orientation of chromosomes (Dimitrave and Gadeva, 1997).

Bridges were the most pronounced type of abnormalities observed in considerable frequencies in both first and second meiotic division and after all different treatments with the three applied concentrations of colchicine. In this type of abnormality one or more bridges were formed between the cell poles. Figure (1) represented bridges in anaphase I with one, two and many bridges. Another bridges showed at anaphase II and telophase II. Chromosome bridges represent one of the most common types of abnormality observed after all periods of treatments with all applied concentrations. This type was produced as a result of breakage and

reunion of broken chromosome ends (Tomkins and Grant, 1972) or due to general stickiness of chromosomes made the separation of daughter chromosomes incomplete and thus they remain connected by chromatin bridges (Abraham and Koshy, 1979).

Multipolar ana-telophase cells were observed after all treatments with colchicine Fig. (1), where the number of poles varies from three to five per cell. In these cells the chromosomes are arranged unequally in three or more poles. Each group will be surrounded by a nuclear envelope forming multinucleate cells Figure (1). Each nucleus had a number of chromosomes lower than the normal diploid one (hyponucleus). The number and size of these hyponuclei per cell are variable from one cell to another. This phenomenon is presumably due to the splitting of the spindle fiber apparatus and accordingly, the set of chromosomes was arranged haphazardly in many groups. Similar results were also obtained by Abdelsalam *et al*. (1993) and Mendes –Bonato *et al*. (2009).

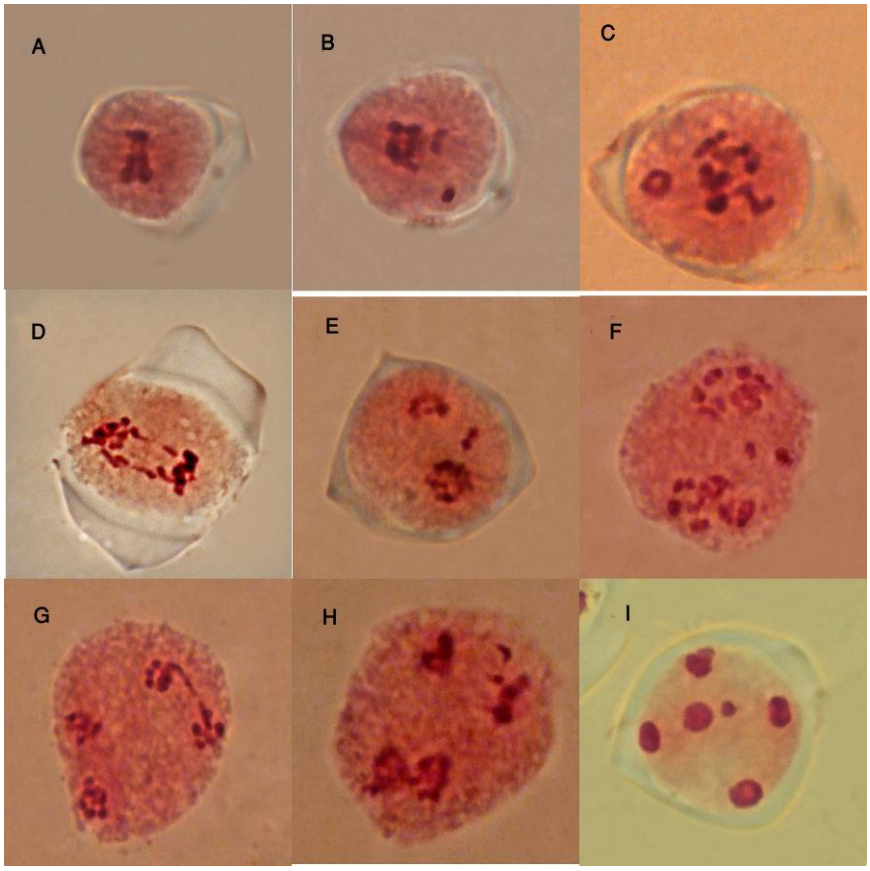

Figure (1): Types of chromosomal abnormalities induced by different concentrations of colchicine: (A) Sticky metaphase I, (B) Sticky metaphase I with two laggards, (C) Metaphase I with ring chromosome, (D) Anaphase I with two bridges, (E) Disturbed telophase I with laggard, (F) Metaphase II with two laggards, (G) Anaphase II with bridge, (H) Disturbed anaphase II with laggard, (I) Multinucleate telophase II with micronuclei.

Among the cytological aberrations appeared in a considerable frequency was the induction of micronuclei Figure (1) showed telophase II with micronuclei. Micronuclei may originate from lagging chromosome or centric fragments. Micronuclei are true mutagenic aspects, which lead to loss of the genetic material and had been regarded as an induction of the mutagenicity of there inducers Gustavino *et al*., (1987) and Ruan *et al*. (1992).

A considerable percentage of metaphase I with bivalents ring was observed in all treatments. Fig. (1) showed metaphase I with one and two bivalents ring, where the sticky ends of bivalents form ring like association at metaphase stage as reported in *Allium cepa* L. by Promilla and Bhattacharya (1991). Also,

McClintock (1941) concluded that ring chromosome originate from a univalent chromosome by non homologous chromosome synapsis of two arms in *Zea mays.*

Stainability of pollen grains was used to record the viable and nonviable pollen grains, stained pollen grain considered a viable one while, the unstained pollen grain is non viable. The percentages of non viable pollen grains increased gradually with increase the concentrations of colchicine until reached to its maximum value of 18.15% at the highest concentration 0.50% as compared with control value of 0.87% Table (3).

The potentiality of colchicine to induce chromosomal abnormalities and reduce the pollen grains viability is similar to the result obtained by Hassan and Ahmed (1999). They concluded that there is a direct relationship between chromosomal abnormalities and pollen fertility, where increasing in the percentages of chromosomal abnormalities reducing the pollen fertility. Other investigators also proved the mutagencity of colchicines. Burun and Emiroglu (2008) stated that colchicine treatment exhibited toxicity to plant cell. Kravchenko (1995) induced artificial tetraploids of the pea by colchicine, the tetraploid plants had low fertility due to meiotic irregularities, such as laggards and bridges. Also, Hassan and Jones (1994) studied the effect of colchicine on the seedlings of *Lolium multiflorum* L., they found that colchicine has long-range effects on chromosome behavior at meiosis. There is a reduction of mean pollen mother cell chiasma frequency and also changes in the pattern of chiasma distribution within bivalents.

In the present study, the detailed cytological observations have shown the occurrence of genetic damage due to colchicine treatments which led to meiotic irregularities and consequently sterility of pollen grains in the flower buds of tetraploid plants of *Nicotiana alata*. So, the meiotic analysis gives a good and reliable genetic assay for screening mutagenicity (Grant, 1994).

The repair effects of the applied nutrients:

Generally, after treatment of tetraploid plants with the three applied nutrients (N, P and K) an obvious decrease in the mean percentages of chromosomal abnormalities were recorded with increasing the rates of the nutrients while, its values still higher than that of the control. Such an inverse relation had been reported by various authors (Ali, 1996 and El-Nahas, 2000). The statistical analysis of the data showed that all the conducted treatments with different rates of the three applied fertilizers had highly significant decrease on the abnormalities of the meiotic division of tetraploid plants previously treated with colchicine as compared with the diploids. The obtained results indicated that, the same types of abnormalities that recorded in the flower buds of the colchitetraploid plants pre treatment with the three applied fertilizers were still noticed after all treatments with them but with different frequencies.

Urea fertilizer:

After treatment the colchitetraploids with different rates of urea fertilizer as demonstrated in Table (2) the maximum mean percentage of total abnormalities was 8.23% after 12 hours treatment with 50 Kg/Fed. as compared with the maximum value of 19.03% after 48 hours treatment with 0.50% colchicin.

Table (3) indicated that the percentage of pollen grains viability had a highly significant increase with increasing the applied rates of urea, where the percentage of non viable pollen grains reached to its maximum value of 10.38% after treatment of tetraploid plants with the lowest rate of urea 50 kg / Fed. while reached to its minimum value of 6.55 % after treatment with the highest rate of urea 150 kg/Fed. as compared with the maximum value of 18.15% with the highest concentration of colchicine after 48 hours treatment.

The obtained results revealed the capability of the urea as a source of nitrogen to counteract the harmful effect of colchicine in the tetraploid plants at cytological level. This capability was explained on the bases of the repairing effect by nitrogen. Where nitrogen is a component of energy-transfer compounds, such as ATP which allows cells to conserve and use the energy released in metabolism. This explanation was supported by Bibi *et al*. (2006) they reported that nitrogen plays a critical role in reproductive growth, especially in the formation of proteins, DNA, and growth promoting polyamines, all necessary for successful fertilization and seed set in cotton. The same results were obtained by Hassan *et al*. (2002) where, they proved the repairing effect of both pre and post treatments with ferty green foliar fertilizer against gokilaht insecticide in *Allium cepa* L. at cytological level. Also, the results were in agreement with (El-Nahas, 2000) who reported that the frequency of mitotic abnormalities induced by pursuit herbicide were decreased after treatment with the herbicide combined with urea fertilizer, and concluded that urea fertilizer had the ability to minimize the effect of the herbicide in *Vicia faba* L. Moreover, Duchovskis *et al*. (2006) found that cadmium in acidic environment produced a very toxic effect on growth, the synthesis of chlorophyll, carotenoids, stem diameter and sap flow rate of

Lycopersicon esculentum. Cadmium suppressed the mitotic index of cells and disorganized normal mitosis, where the mitosis with anomalies (chromosome breaks, fragmentation, bridges, chromosome eliminations and abnormal nucleus divisions) was observed in meristem cells of roots but after treatment with high nutrients rates revealed its repair effect and give favoured plant growth under the effect of cadmium.

Potassium sulphate fertilizer:

The tetraploid plants were treated with three rates of potassium sulphate to determine its repairing effect against colchicine. The meiotic analysis of PMCs in flower buds of these tetraploids revealed low percentages of chromosomal abnormalities after all treatments of potassium sulphate as compared to those untreated ones with the fertilizer. From Table (2) it was seen that, after 12 hours treatment, the maximum value of the percentage of meiotic abnormalities was 10.20 % recorded with rate of 40 kg/Fed. while the minimum percentage of meiotic abnormalities was 4.70 % after 15 days with the highest rates, where the percentage was 19.03% after treatment with the highest concentration of colchicine and 48 hours treatment .

Pollen grains viability after all treatments of tetraploid plants with different rates of potassium sulphate was recorded in Table (3) the frequency of non viable pollen grains was decreased as the rates of fertilizer increased. The maximum percentage of non viable pollen grains was 12.33 % after treatments with the lowest rate 40 kg/Fed. of the fertilizer and reached to its minimum value of 9.28 % with the highest rate 120 kg/Fed. of potassium sulphate fertilizer, as compared with the maximum value of 18.15% after treatment with the highest concentration of colchicine Table (1). From the data presented above, it has been concluded that the cytogenetic toxicity of colchicine can be minimized by potassium sulphate fertilizer. Flowers and Lauchli (1983) concluded that potassium is known to play a vital role in various physiological processes such as meristematic growth, osmoregulation, protein synthesis and long distance transport. These results are in accordance with Hassan (2000) who concluded that the repair effect of stimufol foliar fertilizer after short periods of treatments of *Allium cepa* L and *Vicia faba* L. against nuvacron insecticide is due to the presence of essential compound found in the fertilizer that have a protective mechanism. Also, the obtained result were further reinforced by Shehata *et al*. (1999) they noticed that, the reduction in the ability of colchicine as a mutagen to arrest mitosis in *Vicia faba* L. when combined colchicine solution with water extracts of some medicinal plants, they interpreted the antimutagenic effects of such water extracts against colchicine due to their counteraction effect.

Super phosphate fertilizer:

The obtained data after treatment the tetraploid plants with different rates of super phosphate fertilizer and different treatment times revealed that the percentages of different types of abnormalities were progressively decreased by increasing the applied rates of fertilizer and the time left after the treatment. Table (2) demonstrated the cytological effects of the tested rates on meiotic cell division and recorded a sharp decrease in the mean percentage of abnormal PMCs, at 24 hours after treatment, the maximum value of the mean percentage of meiotic abnormalities was 6.05% after treatment with the lowest rate of 50 kg/Fed. while the minimum mean percentage of meiotic abnormalities was 3.30% after 15 days with the highest rate 150 kg/Fed. as compared with the percentage that was recorded 19.03% after treatment with the highest concentration of colchicine 0.50 % and after 48 hours treatment Table (1).

As indicated from Table (3) which demonstrated the effect of different rates of super phosphate fertilizer on the pollen grains viability. The frequency of non viable pollen grains was decreased as the rates of fertilizer increased. The maximum value 5.42 % was observed at the lowest rate 50 kg/Fed and reached to its minimum value of 3.57% with the highest rate 150 kg/Fed of super phosphate fertilizer as compared with the percentage of non viable pollen grains after treatment with high concentration of colchicine ,it was recorded a maximum value of 18.15% Table (3).

From the previous data recorded after treatment the tetraploid plants with different rates of super phosphate, it can be suggested that the super phosphate fertilizer have the ability to repair the genotoxic effect of colchicine. Similarly, Hassan, (2001) proved the safety of stimofol and nutri-leaf foliar fertilizer and clarified their repairing effect against fenobucarb insecticide of *Allium cepa* L. and *Vicia faba* L. at cytological level. The repairing effect of phosphate fertilizer was proved also by Denlz and Tufan, (1998) where they showed an increase in arms bound and bivalent frequencies in *Meadow fescue* genotypes where the meiotic improvement was realized due to increasing in chiasma frequency which induced by mineral treatment. The same results were obtained by Hassan, (1996) who noticed that black cumin and garlic extracts had

antimutagenic effects with sodium azaide of *Vicia faba* L. Finally, Marschner (1995) supported the result and concluded that an adequate supply of phosphorus is essential to the development of new cells and to the transfer of the genetic code from one cell to anther as new cells are formed and showed the repairing effect of phosphorus. This ability was explained on the bases of super phosphate fertilizer contains phosphorus which is a component of key molecules such as nucleic acids (DNA and RNA), phospholipids and ATP, consequently, plants can not grow without a reliable supply of this nutrient. Phosphorus is also involved in controlling key enzyme reactions and in the regulations of metabolic pathways, in addition phosphorus plays a vital role in virtually every plant process that involves energy transfer. Where, high energy phosphate, held as a part of the chemical structures of adenosine diphosphate (ADP) and (ATP), is the source of energy that drives the chemical reaction within the plant (Theodorou and Plaxton, 1993). It plays an essential role in photosynthesis, respiration and is involved in the formation of sugars and starch. The various role of phosphorus mean it is important in the formation of seeds and development of roots. It also speeds plant maturity and helps the plant resist stresses (Marschner, 1995).

Table (2): Percentage of abnormal PMCs and type of abnormalities of *Nicotiana alata* plants at 12, 24 hour and 15 days after treatment with different rates of (N, P and K).

Duration	Rate Kg/Fad.	Mean % of total abnormal PMCs	% 1St M.D.	% 2nd M.D.	% types of abnormalities						
					sticky	Dist	laggard	bridge	Multi-polar	Micro-nucli	Ring
12 hour	C	0.75±0.10	0.69	0.81	0.13	0.15	0.15	0.08	0.08	0.1	0.1
	50 N	8.23**±0.34	7.31	9.16	25.71	23.68	18.42	13.16	7.09	6.88	5.06
	100N	6.77**±0.26	6.20	7.33	26.11	23.89	16.99	12.56	8.62	6.90	4.93
	150 N	5.58**±0.29	5.20	5.92	28.67	22.99	14.63	12.54	8.96	6.57	5.67
24 hour	C	0.77±0.10	0.74	0.79	0.15	0.12	0.13	0.08	0.07	0.07	0.15
	50 N	7.07**±0.40	7.03	7.1	25.47	20.99	16.51	16.04	8.02	7.31	5.66
	100N	5.58**±0.26	5.02	6.04	27.16	21.49	16.12	14.03	8.36	6.87	5.97
	150 N	4.65**±0.20	4.14	5.09	28.67	21.51	14.34	13.98	8.96	6.81	5.73
15 days	C	0.82**±0.10	0.79	0.84	0.17	0.15	0.13	0.12	0.10	0.08	0.07
	50 N	6.80**±0.47	5.27	8.33	28.43	22.06	17.16	12.26	7.84	7.11	5.15
	100N	4.70**±0.20	3.94	5.7	27.31	24.82	14.89	12.41	8.16	7.80	4.61
	150 N	4.40**±0.21	3.71	4.77	26.89	24.24	15.53	12.88	8.33	7.58	4.55
12 hour	C	0.73±0.07	0.67	0.73	0.17	0.17	0.12	0.10	0.05	0.05	0.03
	40 K	10.20**±0.29	9.08	11.2	27.78	21.08	15.52	13.89	9.31	7.52	4.90
	80 K	8.03**±0.38	7.53	8.47	30.5	22.82	16.81	13.49	6.22	5.81	4.36
	120 K	6.63**±0.36	6.05	7.08	25.63	20.35	16.83	12.56	9.55	6.28	5.03
24 hour	C	0.68±0.06	0.61	0.76	0.15	0.13	0.10	0.08	0.07	0.08	0.07
	40 K	6.48**±0.33	6.55	6.79	23.65	20.57	17.99	14.91	10.54	6.94	5.40
	80 K	6.47**±0.34	6.13	6.4	25.77	21.13	16.75	14.18	10.31	6.70	5.15
	120 K	5.80**±0.25	5.24	6.32	25	20.12	16.67	15.52	10.92	6.61	5.17
15 days	C	0.68±0.09	0.73	0.65	0.15	0.13	0.10	0.08	0.08	0.07	0.07
	40 K	7.00**±0.44	6.42	7.46	28.57	21.67	16.43	11.91	7.62	6.67	5.24
	80 K	5.55**±0.37	5.46	5.62	27.03	26.13	15.32	12.01	8.71	6.61	5.71
	120 K	4.70**±0.31	4.13	5.18	24.82	25.89	15.96	13.48	7.80	7.09	4.96
12 hour	C	0.60±0.11	0.69	0.65	0.12	0.12	0.08	0.10	0.07	0.07	0.05
	50 P	5.87**±0.53	5.02	6.92	2.84	21.02	15.63	14.49	8.52	6.25	5.68
	100 P	4.68**±0.19	4.38	4.47	2.74	21.35	16.37	14.24	7.11	7.12	6.41
	150 P	4.13**±0.28	3.40	4.21	2.7	21.37	13.31	14.92	8.87	7.66	6.85
24 hour	C	0.67±0.11	0.55	0.65	0.13	0.13	0.12	0.08	0.07	0.07	0.07
	50 P	6.05**±0.50	5.32	6.31	2.89	18.73	14.05	14.33	9.64	8.54	5.79
	100 P	4.43**±0.24	3.66	5.74	2.49	24.02	13.54	14.85	8.73	7.42	6.55
	150 P	3.82**±0.29	3.33	4.93	2.65	21.68	12.83	14.16	9.29	8.41	7.08
15 days	C	0.62±0.11	0.56	0.66	0.1	0.12	0.05	0.12	0.08	0.05	0.08
	50 P	4.40±0.33	3.66	5.15	2.69	24.24	15.91	12.5	8.33	7.20	4.92
	100 P	3.75±0.18	3.50	3.98	2.62	24.44	12.44	13.79	9.78	8.00	5.33
	150 P	3.30±0.11	3.40	3.21	2.53	25.76	12.63	15.15	10.61	8.59	5.05

* Significant from control at 0.05 level (*t*- test) ** Significant from control at 0.01 level (*t*- test)

Table (3): Percentage of nonviable pollen grains of tetraploid plants of *Nicotiana alata* ,induced by colchicine then treated with different rates of urea, potassium sulphate and super phosphate.

Material	Rates	Percentage of non viable pollen grains/plant						Mean% of Nonviable pollen grains± SE
		1	2	3	4	5	6	
Colchicine	C	1.20	1.00	0.80	0.70	0.60	0.90	0.87 ± 0.10
	0.10	10.00	12.00	11.00	12.00	13.00	9.50	11.25** ± 0.54
	0.25	18.00	17.90	15.90	16.90	17.50	17.50	16.68** ± 0.64
	0.50	21.00	17.00	19.50	16.10	17.30	18.00	18.15** ± 0.73
Urea	C	0.50	1.00	0.90	0.70	0.60	0.80	0.75 ± 0.11
	50	9.20	12.00	9.00	10.00	10.80	11.30	10.38** ± 0.49
	100	8.80	9.70	7.60	9.50	11.10	10.00	9.45** ± 0.48
	150	5.50	6.00	7.90	6.80	8.00	5.10	6.55** ± 0.50
Potassium sulphate	C	0.90	0.60	0.50	1.00	0.70	0.70	0.73 ± 0.16
	40	12.00	13.00	12.60	14.00	12.00	10.40	12.33** ± 0.49
	80	9.00	11.00	13.00	12.50	11.50	10.90	11.32** ± 0.57
	120	9.90	10.80	7.10	9.80	10.10	8.00	9.28** ± 0.58
Super phosphate	C	0.90	0.80	0.30	0.50	1.10	1.00	0.77 ± 0.13
	50	5.30	4.50	6.00	4.90	6.80	5.00	5.42** ± 0.34
	100	4.10	4.30	4.40	4.50	4.90	5.20	4.57** ± 0.17
	150	3.30	3.50	3.60	4.00	3.80	3.20	3.57** ± 0.12

* Significant form control at 0.05 levels (*t*- test) ** Significant form control at 0.01 levels (*t*-test)

4. Conclusion:

Concerning the effects of the three applied nutrients urea, potassium sulphate and super phosphate on the flower buds of tetraploid plants of *Nicotiana alata*, it was concluded that the three nutrients had the ability to counteract the cytotoxicity of colchicine, where the super phosphate fertilizer more effective to counteract the mutagenicity of colchicine and revealed its repairing effect at cytological level than urea and potassium sulphate fertilizers.

Corresponding author
Elham A. A. Abd El-Hady
Department of Genetics and Cytology, National Research Center, Dokki, Giza, Egypt
elhamabdelhady@hotmail.com

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