

Effects Of *Rhazya Stricta* Leaves Extract In Root Tip Meristems Of *Vicia faba*

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Abstract: Higher plants used extensively in traditional medicines are increasingly being screened for their role in modulating the activity of environmental genotoxicants. *Rhiza stricta* (*R. stricta*) Decne is small glabrous erect shrub with a smooth central stem and dense semi-erect branches which grows commonly in the Arabian Gulf region and the Indian subcontinent. This study has concerned with the answer of an important question: what is the effect of the leaves of *R. stricta* water extract of genetic effect or no? and if there is a genetic effect is it positive or negative or in another word the leaves of *R. stricta* water extract cause a decrease or an increase in the activity of environmental genotoxicants. To determine the effect of *R. stricta*, plant extracts from fresh leaves were prepared using liquid nitrogen. The seedling of *vicia faba* will be treated with *R. stricta* extracts at different doses (5, 10, 20, 30, 50, 150, 200g/l) and different intervals. The result of the last seven treatments can be summarized as follows: In all treatments have lead to the increase of mitotic index, when compared with the control. Also, in all treatments have lead to the increase of mutation frequency, when compared with the control. In addition, in all treatments have lead to a decrease of normal mitotic stages, when compared with the control. Moreover, in some treatments have lead to a decrease of mutation mitotic stages, and some treatments have lead to a decrease of mutation mitotic stages, when compared with the control. All treatments have caused different kinds of mitotic abnormalities and chromosomal aberrations, which were generally as follow: change percentage of mitotic phases, C-Metaphase, Stickiness, Break and Fragments, Bridges, Tripolars, Polyploidy, Rings, Binucleates, and disturbance. In single treatment with heavy metal the highest percentage of aberration (41.87%) appeared after the treatment with (30g/L), and the most frequent kind of these aberrations was Break, fragments (76.91%), and little frequent kind of these aberrations was Polyploidy(0.6).

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Key word: *Rhazya Stricta*, *Tip*, *Meristems*, *Vicia*, *Faba*, *Genetics*

1. Introduction:

Rhazya stricta, an evergreen poisonous under shrub, has covered large small hill of District Karak, Pakistan. *R. stricta* like other plants is competing with the main crops for nutrients and other resources and hamper the healthy growth of crops ultimately, reducing the yield both qualitatively and quantitatively.

Allelopathy refers to the beneficial or harmful effects of one plant on another plant, both crop and weed species, by the release of chemicals from plant parts by leaching, root exudation, volatilization, residue decomposition, and other processes in both natural and agricultural systems (Gibson and Liebman, 2003). Allelopathy expected to be an important mechanism in the plant invasion process because of the lack of co-evolved tolerance of resistant vegetation to new chemicals produced by the invader. The definition is sometimes broadened to include non-antagonistic relationships between organisms. In agroecosystems, allelopathic effects between living weeds and crops, crops in mixtures, plant straw residue and succeeding crops during decomposition of residue, (Rice, 1984 Baeshin *et al.*, 2005, 2008, 2009 and 2010, Khan *et al.*, 2011).

This phenomenon could allow the new introduced species to overlook natural plant communities. Allelopathy is the production and release of chemicals that harm or otherwise decrease the fitness of other plants (Hierro and Callaway, 2003). Allelopathy was also studied by many researchers (Alford *et al.*, 2009; He *et al.*, 2009; Thorpe *et al.*, 2009). Ahmad *et al.*, (1983) and Al-Yahya *et al.* (1990) have reported the presences of alkaloids, glycosides, triterpenes, tannins and volatile bases in the leaves of this plant. To explore allelopathic potential of *R. stricta* we examined the effect of aqueous extract of leaves and stem of this plant on seed germination and seedling growth of *Zea mays* species which come with rain water to the field and growing naturally together with *R. stricta* (Baeshin *et al.*, 2005, 2008, 2009 and 2010, Khan *et al.*, 2011).

The objective of this study of genetic effects of *Rhazya stricta* leaves extract In root tip meristems of *vicia faba*. Also, this study has concerned with the answer of an important question: what is the effect of the leaves of *R. stricta* water extract of genetic effect or no. In addition, if there is a genetic effect is it positive or negative or in another word the leaves of

R. stricta water extract cause a decrease or an increase in the activity of environmental genotoxicants.

2. Materials and Methods

1- *Rhazya stricta* plant

It was collect from the nearby areas of Jeddah, KSA. The leaves were wash shade-dried and ground to a fine powder with a blender and the resulting powder were diluted in distilled water. Leaves Are collected and tops of developing soft and dark green in the period between the months of November to the end of January. Which is the best time in the life cycle of the plant where the Mkhaddra and soft too, where the selected sampling plant and took her leaves and tops of developing soft from the buffer zone between Mecca and Taif called the anemone, brings together specific weights of leaves and soft peaks developing in the glass cup.

2 The work of plant extracts:

Attend the weights of specific securities and summits developing soft plant rue, then placed in a dish mortar sterile, then pour the liquid nitrogen. Which leads to stiffness (dried) leaves and tops of developing because of the severity of cold, and then begin the process of grinding is grinding papers and summits developing until it reaches the form of powder. Then add to the specific sizes of distilled water sterilized by concentrations required, with the work of seven different concentrations are (5 g / liter, 10 g / liter, 20 g / liter, 30 g / liter, 50 g / L, 150 g / liter, 200 g / l), then leave these concentrations for 7 days at a temperature of cold (refrigerator). Then filtered using a centrifuge speed 1500 r / min, or Recommended by the suppression of glass sterilized and covered with several layers of gauze (Cheese Cloth) and sterilized to get rid of the plant parts Alrasbh.

3 - Plant the seeds of faba bean:

Used the seeds of a plant faba bean sound and free from injury of State and decay, which was obtain from the College of Agriculture - Cairo - Arab Republic of Egypt. It has applied during the transactions of the recommendations mentioned by **Kihelman 1975** to prepare the tops of the roots and to study the effect of transactions chemicals on chromosomes.

4 - Genetic Alcetologih preparations:

Soaking Soaking

Soak the seeds of good non-infected beans for 6-12 hours in tap water, taking into account the size

of the pot so that it is appropriate for the number of seeds, and water is a little chlorine, and oxygen-rich and change periodically, with the water temperature ranging between 20-22 degrees Celsius.

Germination

After soaking the seeds allowed to Balanbat between two layers of cotton soaked for 4 days at 22 ° C, after this initial period will grow roots (Primary roots) a length of 3-5 cm and thus movement of seedlings to a suitable container by the water faucet.

Third, the period of growth in the water Growth period in water movement of seedling in a pot with water renewed its temperature around 20 degrees Celsius for 24 hours can be used in the primary roots of various transactions. Dye Staining Preparation of slides Preparation of slides, Examination of the slides (Scanning of slides), applicable laws in the trials according to Baeshin 2005, 2008, 2009, 2010 and Khan *et al.*, 2011.

5 - Statistical methods:

Different results were subject to statistical analysis using the biological testing method independent samples T (Independent-Sample T-Test). It was to assess for significant concentrations of the process control (water concentration) to see significant differences between the averages of pairs of transactions.

3. Results

The cytogenetic effects of the transaction Palmstkhals aqueous leaf Rue on the peaks of the roots of developing faba bean plants

Results shown in Tables (1-2) effects resulting from the treatment cells construction of the roots of a plant faba bean seven different concentrations of extract of leaves Rue (5, 10.20, 30, 50.1502 million g / liter). Have led to this treatment affected the proportion of mitotic directory (Mitotic index) and the frequency Alotfora (Mutational frequency) and the percentage of phases Almitoseh (Mitotic stages) and the ratios of different types of chromosomal imbalances (Chromosomal Aberrations) compared to the control.

As shown in Table (1): that different concentrations of aqueous extract of leaves Rue have had insignificantly increased in the ratio of directory mitotic and in the concentrations of the following gave the concentration (5.10, 20, 50.1502 million g / l) the following ratios (14.35, 16.77, 14.04, 14.73, 17.10, 17.68%). The concentration (30 g / L) has caused a significant decrease in the proportion of the directory where the mitotic rate (11.75%). As compared to the control

Table (1): The percentage of the guide in the mitotic division cycle in cells indirect construction of faba bean plants after treatment with different concentrations of aqueous extract of the *Rhazya Stricta* plant

Indicator of mitosis	Total cells	Total cells	concentrations
12.47	532	4265	Control
14.35	597	4161	5g
16.77	677	4036	10g
14.04	584	4159	20g
11.75*	492	4188	30g
14.73	600	4074	50g
17.10	696	4071	150g
17.68	716	4050	200g

Table (2): Analysis of the percentage of the mitotic cycle of manual division indirect construction in the cells of faba bean plants after treatment with different concentrations of aqueous extract of the *Rhazya Stricta* plant

Sig. (2-tailed)	df	t	Paired Differences				Water con.	Pair	
			95% Confidence Interval of the Difference		Std. Error Mean	Std. Deviation			Mean
			Upper	Lower					
.825	2	.251	224.5108	-199.7641	49.30388	85.39683	12.3733	Water con. 5	Pair 1
.830	2	.244	495.8226	-442.6893	109.06201	188.90094	26.5667	Water con. 10	Pair 2
.744	2	.375	217.9557	-183.0024	46.59428	80.70367	17.4767	Water con. 20	Pair 3
.217	2	1.782	133.9991	-55.5191	22.02342	38.14568	39.2400	Water con. 30	Pair 4
.656	2	.518	374.6873	-294.1940	77.72894	134.63047	40.2467	Water con. 50	Pair 5
.942	2	.082	454.0077	-437.0944	103.55264	179.35843	8.4567	Water con. 150	Pair 6
.947	2	.075	505.0714	-487.8781	115.38807	199.85799	8.5967	Water con. 200	Pair 7

The results were shown increase in the proportion of repetition Alotfora any repetition of the prevalence of imbalances aberrations in cells of construction of the roots of a plant faba bean and treatment Baltrakiz different from the extract of leaves Rue, where the given concentrations of the following (5, 10, 20, 30, 50, 150, 200 g / l). The following ratios were (0.39, 0.32, 0.34, 0.42, 0.41, 0.35, 0.40 %) As compared to the control where the ratio is (0.26%), as shown that the less the effect of the extract plant is when you concentration (10 g / l) where the ratio was (0.32%) while the highest impact of the plant extract is at a concentration (50 g / l), where the ratio was (0.41%).

Also the results shows the effect of treatment Palmstkhals aqueous leaf Rue on the percentages of cells Almitoseh, have resulted in treatment Baltrakiz different to a general decline in the percentage of cells Almitoseh compared to the control, where the concentration (5, 30, 50, 200 g / L) was significantly lower ratio is (61.48, 58.14, 59.00, 59.64%). Is clear

from the an increase in the proportion of repeat introductory phase where he was the highest increase at a concentration (10 g / l), where the percentage (48.60%), while in the other various concentrations has caused an increase in the proportion of recurrence compared to the preliminary phase to the control. The metaphase cells have happened to her in all the lower concentrations where the rate of decline was greatest at a concentration (200 g / l), where the ratio was (1.96%). Phase cells, while the separatist has happened to her in the proportion of low frequency, where the greatest percentage decrease at a concentration (30 g / l), where the ratio was (4.27%) compared to the control. Telophase cells as well as had happened to her in the proportion decrease in frequency, and increasing at a concentration (30 g / l), where the ratio was (8.54%) compared to the control.

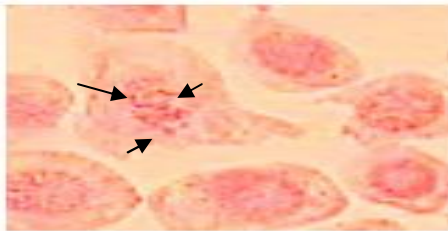
It is clear the treatment of cells of the roots of a plant faba bean construction Palmstkhals aqueous leaf Rue lead to increased non-significantly in the proportion of phases Almitoseh dysfunctional

abnormal compared to the control at concentrations (5, 30, 50 200 g / l). Where The ratios (38.53, 41.87, 30.83, 29.71%). Whereas at a concentration (10 150 g / liter) has led to a significant decrease in the ratio (20.63, 23.96%) as Figures (8-11) a decrease in the proportion of repetition of the preliminary phase unbalanced in most concentrations. Whereas at a concentration (200 g / L) occurred in the proportion of high frequency phase unbalanced primer where the ratio was (4.75%) compared to the control. While metaphase cells dysfunctional, there has been a general decline in the proportion of repetition, but the increase occurred at a concentration (5.30 g / L), where the ratio was (12.23, 8.33%) compared to the control. The separatist-phase cells dysfunctional, there has been a high increase in the rate of repetition at all concentrations, and was most at a concentration (30 g / l), where the percentage (24.80%) compared to the control. As well as cells telophase dysfunctional has happened to increase at a concentration (30, 50 150 g / l), where the ratio was (6.10, 7.33, 6.75%) and a decrease at a concentration (5, 10, 20 200 g / l), where the ratio was (4.19, 4.87, 5.31, 3.63%) compared to the control.

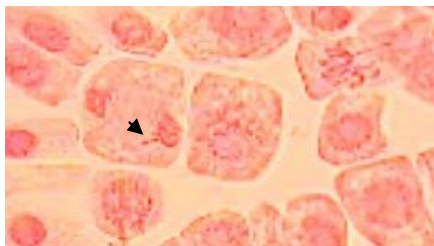
The ratio and the types of imbalances, chromosomal Almitoseh resulting from the transaction Palmstkhals aqueous leaf Rue compared to the control were represented these imbalances in Pictures bridges chromosomal (Bridges). the reluctance of chromosomal (Laggards), the viscosity of chromosomal (Stickness), dispersion of chromosomal (Desturbance), fractures and fragments of chromosomal (Breaks and Fragnmnts), a tropical Kulchesina (C-Metaphase). the leaders of three (Tripolars), chromosome ring (Ring Chromosomes), nuclei minutes (Micronucli), multi-group chromosome (Polyloidy), as shown in the figure (1).

Recorded the highest percentage of imbalances at a concentration (30 g / l), where the percentage (41.87%) and lowest at a concentration (10 g / l), where the percentage (20.63%) compared to the control.

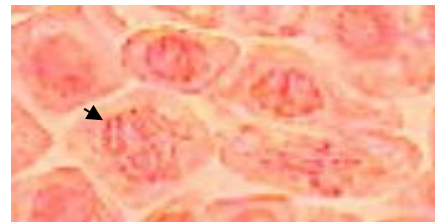
Has also recorded the highest proportion of fractures at the types of imbalances and chromosomal fragments, where the ratio was (76.91%), and the lowest was at the multi-group chromosome, where the ratio was (0.68%).



Break and fragments chromosome



Reluctance of chromosomal



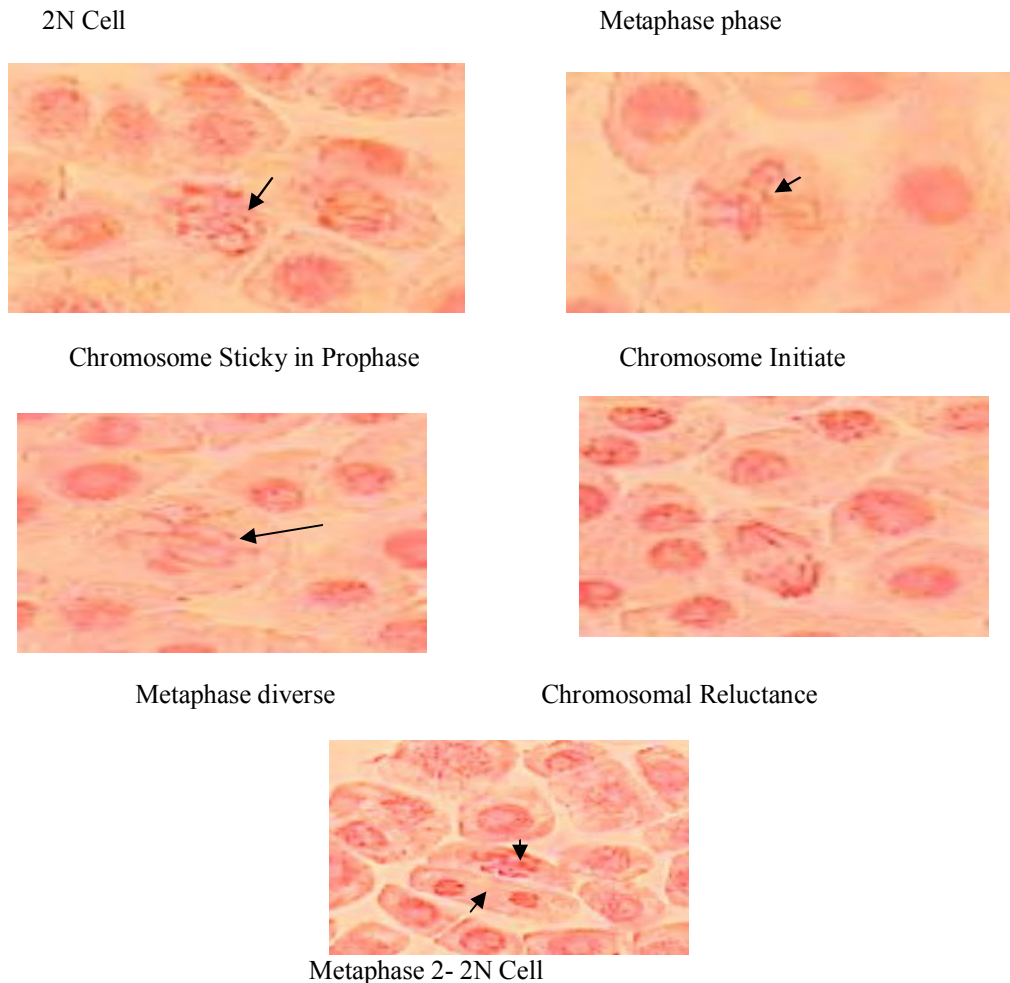
Duplicate Chromosomal Group



Bridge chromosomal



Figure 1. Examples of chromosomal imbalances in mitosis resulting from treatment with different concentration of aqueous extract of the *Rhazya Stricta* plant



4. Discussion

The cytogenetic effects of the transaction Palmstkhals aqueous leaf Rue on the peaks of the roots of developing faba bean plants. Been testing the impact of seven concentrations (5, 10.20, 30, 50.1502 million g / L) of the aqueous extract of the plant Rue on the proportion of directory mitotic peaks developing the roots of faba bean as is clear increase the percentage of directory mitotic of concentration to another. Also, when compared to the control. Where the concentration was (30 g / L) significantly affected the decline in the proportion of directory mitotic. While the rest of the concentrations were the impact is significant in increasing the percentage of directory mitotic, and shows that it is an increase of concentration used is high mitotic directory. It also increases the frequency Alotfora for the number of cells overall, but it did not show significant differences at any concentration of different concentrations, also led various transactions to the low percentage of cells Almitoseh, and had a concentration (5, 30.50 0.200 g / L) significantly affected, while the concentration (10, 20 150 g / L)

effect was not significant. Treatment also led to increase the percentage of defective cells at specific concentrations, where the focus (5, 30, 50 200 g / L) led to an increase not significant, and the concentration (10 150 g / liter) led to moral decline. The treatment also led to the emergence of different types of chromosomal imbalances. The results were obtained from the different treatments can be explained by many of the studies carried out to study the characteristics of natural compounds. Including the study of the chemical composition of leaves Rue, which showed that the plant Rue is characterized by some of the qualities that have much to bring about the effects of which are: The plant extract contains two types of chemicals are Algulwadiat and Filavonyudat, Atta, *et al.*, 1989. The plants extract works to reduce motor activity in mice, Ali, *et al.*, 1995. The plant extract is an anti-oxidant, Ali, *et al.*, 2000. Plants extract that works to increase the proportion of enzyme (AST) and high-density lipoproteins and Albulsiroben, urea, and a decrease in concentration of albumin, calcium, Adam 1998. The plant extract may serve to reduce the concentration of

amino acids, cerebral, Ali, *et al.*, 2000. Plant extracts that works to increase the concentration of glutathione, and reduce the concentration above the oxidizing fat and ascorbic acid. Ali, *et al.*, 2000. Plant extracts that acts as a toxic by Djintamesin, Ali, *et al.*, 2002. Plant extracts that works to increase the isotopes Alsaitokrom P-450, EL-Kadi *et al.*, 2003.

These properties may explain the reason for the high percentage of mitotic directory. Noting increased cell stage primer, which is due this accumulation to the influence of plant extract on the spindle apparatus and thus the effectiveness of yarn spindle so that it fails to do its job properly. This result is similar to the impact of plant extract fittings medical, Adam and Rashad, 1984, Baeshin *et al.*, 2005, 2008, 2009 and 2010, Khan *et al.*, 2011.

It is clear in Fig 1 types of chromosomal imbalances resulting from the transaction Almitoseh different concentrations of aqueous extract of the plant Rue, where the concentration (5.10, 20, 50.1502 million g / liter) and that of the most common. Chromosomal Fragmentation, note the existence of this type of chromosomal imbalances in large quantities in this study, especially at the end of the preliminary phase and metaphase, the presence of chromosomal pieces resulting from the broken chromosomes, or may note the presence of cut Kromatidih caused by fractures in the chromatin. The reason for this treatment of the cell fractions to one of the factors of chemical or physical mutagens that interfere with the links in the molecule of DNA, or may result in breakage of the severe vibrations, especially if the sample came in a mashing and mashing process was intense. This situation of confirmed cases of genetic toxicity, as they are, during which the loss of part of the genetic material carries genetic information specific to the relevant neighborhood. This is what was observed in several studies Alcetologih, including study Lechardeur, *et al.*, 2004, Caddick, *et al.*, 2005, Castiglia, *et al.*, 2005, Baeshin *et al.*, 2005, 2008, 2009, 2010 and Khan *et al.*, 2011.

Chromosomal Bridges, As obtained in this study on the chromosomes or Kromatidat stuck with each other, resulting in a fusion between Kromusuman or Kromatidtan separatist phase, appears in the form of bridge (or bridges) or chromosomal Kromatidih. Where we find that, despite the entry of the division in developed separatist, and that it should be separated into two identical chromosomes at the poles of the cell, we find there are some identical chromosomes are still welded to each other in terms of the parties until the end of the separatist phase. He developed this state of bridges, chromosomal or Alkromatidih in several studies Alcetologih, including those noted by on onion plant to test

genotoxicity of pesticide Fayyoradan, resulted in this case is connected to the chromosomes in the form of chromosomal bridges, Stewenius, *et al.*, 2005;., Gisselsson, and. Höglund 2005; Shimizu *et al.*, 2005; Luo, *et al.*, 2004.

Chromosomal Lagging notes the existence of this type of imbalances chromosomal large amount in this study. Where find in this case that one of the chromosomes or more different from the rest of the chromosomes in the movement to a polar cell. Would stay longer at metaphase because of the cell displays at this time to a chemical agent or physical impact on the movement of the spindle threads related chromosome, or for other reasons are not known. Have been observed this case in several studies, including: the study of Rocha *et al.*, 2004; Stewenius, *et al.*, 2005. Some of these chromosomal imbalances is a clear indication for a genotoxic chromosomal level, which are unlikely to have a role (effect) Fore at the genetic level, which requires extensive study (detailed) at the molecular level of genetic material. Chromosomal dispersion has been seen in the case of scattered chromosomes (irregular) in this study, especially in the metaphase of division and separatism indirect. Have been watching this dispersion and irregular chromosomal in several studies Alcetologih including, study Gisselsson, and. Höglund, 2005 and there are many other studies that have shown this type of chromosomal imbalance. This case has appeared in several studies, including, study Muller *et al.*, 2005. The pairs of chromosomes (Stickiness): As obtained in this study on the chromosomes is not clear-cut, has appeared interoperate with each other, suggesting that like a single block. Has been seen in several studies, including: study Bush, *et al.*, 2005; Aguado *et al.*, 2005, Baeshin *et al.*, 2005, 2008, 2009, 2010 and Khan *et al.*, 2011.

Conclusion

Root tips of *Vicia faba* were treated with *Rhyza stricta* plant extract from fresh leaves, The result of the last seven treatments (5, 10, 20, 30, 50, 150, 200 g/l). can be summarized as follows: This study has con-cerened ware tok care of the answer of an inportant cooestaon waths is das the leaves of *R.stricta* water extract of genetic effect or no ? and if there is a genetic effect is it postive or negative or in another word the leaves of *R.stricta* water extract cause a decrease or an increase in the activity of environmental genotoxicants.

To determine the effect of *R.stricta* , plant extracts from fresh leaves were prepared using liquid nitrogen . The seedling of *vicia faba* will be treated with *R.stricta* extracts at different doses, can be summarized as follows:

All treatments have lead to the increase of mitotic index, in singl treatment with the increase of mitotic index (17.68%) appeared after the treatment with (200 g/l), when compared with the control. All treatments have lead to the increase of mutation frequency, in singl treatment with the increase of mutation frequency (0.42%) appeared after the treatment with (30 g/l), when compared with the control. All treatments have lead to a decrease of normal mitotic stages, in singl treatment with a decrease of normal mitotic stages (58.14%) appeared after the treatment with (30 g/l), when compared with the control. some treatments have lead to a decrease of mutation mitotic stages, in singl treatment with a decrease of mutation mitotic stages (20.63%) appeared after the treatment with (10 g/l), when compared with the control, and some treatments have lead to the increase of mutation mitotic stages, in singl treatment with the increase of mutation mitotic stages (41.87%) appeared after the treatment with (30 g/l), when compared with the control. All treatments have caused different kinds of mitotic abnormalities and chromosomal aberrations, which were generally as follow: change percentage of mitotic phases, C-Metaphase, Stickiness, Break and Fragments, Bridges, Tripolars, Polyploidy, Rings, Binucleates, and disturbancee. In single treatment with heavy metal the highest percentage of aberration (41.87%) appeared after the treatment with (30g/L), and the most frequent kind of these aberrations was Break, fragments (76.91%), and little frequent kind of these aberrations was Polyploidy(0.68%).

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