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Research Article

The possible cytogenecity and mutagenicity effects of *Allium sativum* as a natural fungi/ pesticide on faba bean plant

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Abstract

Allium sativum (garlic) is one of the most famous plant rich in Sulphur products which are so beneficial in bio-gardening and agriculture if applied in right ratios as natural fertilizer to the soil poor in organic matter (under 2%) or applied as natural fungicide &pesticide. It like many other important folk plants needs further investigations and revising for its products' cytogenecity and mutagenicity. This study was designed to highlight the mode of action of fresh garlic water extract against two other garlic products (powder and tablets) dissolved in water on faba bean plant, as an ideal model system for both cytogenetic and biochemical investigations. Tomix tablets in water (two and three tablets/L), garlic powder (400 and 600 mg/L) and aqueous extract of smashed fresh garlic cloves (4500 and 7110 mg/L) were examined for their effect on plant mitosis and protein profile after 24 and 48hr of treatments. The obtained results showed that; treatment with each form of the three tested garlic products induced concentration dependent reduction in the mitotic index, and affected notable change in the mitotic phase' frequencies. Its effect on chromatin material is much greater than its effect on spindle formation or chromosomes mobility in cytoplasm. It also affected the proteins profile of the treated seedlings using SDS-PAGE.

Conclusion: Treatment with fresh garlic and with garlic powder shows concentration dependent cytogenecity and mutagenecity. Prolongation of treatment time to 48hr increases the clastogenecity risk. Recommendation: the usage of fresh Garlic extract as a fungi/ pesticide in agriculture should be with caution, as it show some mutagenicity and cytogenicity.

Introduction

Some of plants' derivatives (ashes, extracts, oil, powder and pasts) have been admitted as effective natural defense system to other plants against biological pests and pathogens in an eco-friendly way.

These derivatives can keep the environment cleaner, less hazardous and healthier if compared with other chemically synthesized pesticides, herbicides or fungicides; which found to be a real threat to the air, soil, irrigation canals and wells as they are not easy to be degradable. Moreover, the extensive usage of these chemicals found to enhance the pest's resistance year over year (ISU Extension and Outreach and North Central IPM Center for financial support; guide flyer) and may cause a serious deterioration in plant and crop quality. Thus, inspire the researchers to seek for natural active pesticidal and or fungicidal (plant products& preparations) and re-consider this natural gift and revise it on many levels to declare its effectiveness and safety.

The cytogenetic level and gene expression level come to the front of those levels of revising. For example; natural pesticides such as *Sorghum bicolor* seedling water extract and petroleum ether extract of *Nasturtium officinale* seedlings' extract which can control the cotton leaf warm and the seeds powder of *lupinus termis* and black cumin which can protect the stored seeds from bruchids' attack; were all studied and revised for their possible cyto-genecity and mutagenicity on *Vica faba* plant [1,2]. Some of plants' essential oils are already marketed as fungicides for bio-gardening such as. "E-Rase™" from jojoba oil, "Sporan™" from rosemary oil, "Promax™" from thyme oil, "Trilogy™" from neem oil and "GC-3™" being a mixture of cottonseed oil and garlic Allium sativum [3].

Among the promising natural product is Garlic (*Allium sativum)* which known by the therapeutic effect of its derivatives

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in agriculture as it contains an abundance of chemical compounds such as organophosphorus and Allicin compounds that have been shown to possess beneficial effects to protect against several plant pathogens [4].

Garlic (*Allium sativum* L., order Asparagales, Family Liliaceae) is a widely distributed plant in our region; it is herbaceous plant with height of 20-40 cm, a bulb of strong odor and pungent taste. It is one of the most important multi purposes used plant.

The active ingredient Allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate) is considered as a defense molecule and the principal bioactive compound present in the aqueous extract of garlic or raw garlic homogenate. When garlic is chopped or crushed, allicin is rived from alliin by alliinase enzyme (a CS-lyase, E.C. 4.4.1.4) [5].

Allicin in fresh garlic has an antibacterial effect on gram-positive antibacterial and gram-negative bacteria [6], also an anti-fungal activity when applied to seeds before planting [7,8] and can be used as natural pesticide controls nematodes in ground nuts, flower thrips (WFT) in strawberry, mildews in cucumber, tomato leaf miner, leaf blotch in cereals [9]. Allicin is suggested to have toxic effect as it is readily membrane permeable [10], and easily enters cells and reacts with cellular thiols such as glutathione [11] or cysteine residues in proteins [12]. As a consequence, enzymes with accessible reactive cysteines can be affected in their function [13].

So based on this context, garlic is considered as a very important plant with great beneficial rule on many other plants' protection against pathogens and pests. It may contribute to the development of natural products for the agricultural and other industrial uses as crud past, extract, it may be dried into powder or it may be used as building blocks' necessary to synthesize more complexed form such as medicinal tablets.

Study the mitotic division and phases index and frequency can give a clear image about the chromosomal behavior, segregation and transition of the genetic materials in-between the daughter cells in growing plants. Examine the bioactive natural products on model system meristem's cells reflect its mode of action on the mitotic apparatus [14].

Also, protein profiling of seedlings' total protein of model plant under treatment with examined material refers to the condition of gene expression into proteins.

The aim of this research is to visualize the cytogenetic and biochemical (SDS-PAGE) effects of *Allium sativum* (garlic) in three different forms (mashed fresh cloves, garlic powder and medicinal tablets) dissolved in water on *Vicia faba* L. (Giza 716 Egyptian cultivar) as plant model system.

Materials &methods

The designed experiment was carried out on bench top in genetics and cytology lab, NRC, Egypt.

I- Plant & experimental materials

The *Vicia faba* (2n = 12) (Var. Giza 716); used for all the conducted experiments, were obtained from the Crop Research Institute, ARC, Giza, Egypt. And then germinated on filter paper rolls in a large beaker with 3cm height tap water at the bottom and divided into two groups for two designed experiments.

Tomix tablets (a natural medicinal preparation depends on garlic as building block) were purchased from pharmacy under commercial name "Tomix". The used doses are 2 &3 tablets/ litter , Garlic powder (an organic food additive) was purchased from a grand store of foods under commercial name "isis", the used concentrations were 400 & 600 mg /L which are calculated to be equivalent to the garlic ingredient in Tomix tablets and Garlic fresh raw (fresh aqueous extracts) were prepared after [6] in the lab as following: two weighs (two cloves ~ 4.5gm& three cloves ~7.11gm) of garlic were crushed and smashed into fresh paste in clean mortar and then dissolved in 1L-as it is the best way to extract allicin from garlic.

II- Treatment with the tested materials

Experiment for cytological studies: About 2 cm length main roots of *Vicia faba* were treated for 24 & 48hrs with the experimented materials for each concentration at room temperature. Treated roots were then fixed with Carnoy's fixative for 24 hr before preservation in 70% alcohol. Slides were prepared for cytogenetic investigation [15]. Effects of examined chemical treatments and control on different chromosome plates were observed under Olympus light microscope. To determine the effect on mitotic index, 2500-3000 cells were scored in control group and in each treated sample.

Mitotic indexes and Percentages of cells showing chromosomal abnormalities and type of abnormality were recorded at the appropriate mitotic stages. Mitotic index were calculated by this formula:

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells observed}} \times 100$$

Experiment for protein electrophoresis group: After the seeds were germinated and grow to root, shoot and leaves, whole seedling plant of *Vicia faba* were treated for 24& 48hrs with each of the experimented materials in two concentrations at room temperature.

Total proteins were extracted from the treated plant's leaves to perform sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli [16] and as described in Tsugama, et al. [17]. By grinding 1g of leaves and mixed with 1 ml of extraction buffer in eppendorf tube and left in refrigerator overnight, then vortexed for 15 seconds and centrifuged at 12,000 rpm at 4˚C for 20 min. The supernatants containing total proteins were transferred to new eppendorf tubes and kept at deep-freeze until use for electrophoretic analysis. The marker of the used protein is BLUltra Prestained Protein Ladder (GeneDirex, Cat No. PM001-0500). In this method, 10% protein separating gel were used. Protein fractionations were performed on vertical slab gel (19.8 cm x 26.8 cm x 0.2 cm) using the electrophoresis apparatus manufactured by Cleaver, UK. The images were captured by

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digital camera (Sony, made in Japan) and transferred directly to the computer and then the protein bands were analyzed by Total Lab program to find out the molecular weight of each band and that to find the effect of each treatment on gene expression of different genes responsible for the formation of proteins in faba beans.

Results

The data shown in (Tables 1,2) revealed that: 24hr treatment, each form of the three tested garlic product induced concentration dependent reduction in the mitotic index at root tip meristems of *Vicia faba* relative to the control, it also reveals that, the aqueous extract of garlic fresh cloves followed by the garlic powder in their lower concentrations score the highest mitotic index among the tested materials; this was reversed by increasing their concentration as they score the lower mitotic index which means; this form of garlic product at the higher concentration are the most destructive as they have the higher cytotoxic effect (Table 1).

It worth to mention that, although the cytotoxic effect was prolonged by expanding the exposure time of treatment to 48hr, there was a notable improvement in the mitotic indexes recorded after the higher concentration of Garlic in the tested forms (3cruched Tomix tablets 8.03%, 600mg Garlic powder 6.3%) and nearly with no effect on mitotic index after 7.11gm/L Garlic cloves (Table 2).

Garlic products affected notable change in the frequency of mitotic phases (Figure 1), mostly at prophase stage, as all forms prolong the prophase stages relative to control. As illustrated, 24 hr treatment with (4.5gm/L of cloves aqueous extract showed the biggest effect on prophase stage 53.38% followed by the 600mg/liter of garlic powder 45.45%. on the other hand treatment with three tomix tablets induced the highest effect on metaphase frequencies reaches 45.21% on response of ana-telophase duration which was 15.75% . Regarding the ana-telophases garlic products in the three tested form alter the ana-telophases by lowering its index; except after the 400mg/liter of garlic powder as it scored higher percentage if compared with control. Regarding the effect on both prophaeses and metaphases, 24 hr treatment with three tablets of tomix induce the highest effect on prophase and metaphase frequencies reaches 39.04& 45.21% on response of ana-telophase duration which was 15.75% followed by treatment with 4.5gm/L of garlic cloves induce the highest effect on prophase and metaphase frequencies reaches 53.38, 21.43% on response of ana-telophase duration which was 25.19% as shown in Tables 1,2 and Figure 1.

Table 1: Mitotic index, percentages of abnormal mitosis and abnormality in each mitotic phase in *Vicia faba* root- tip meristems, after 24&48 hrs of treatment with extracts of three different Garlic form.

Treatment	Concentration	Time for treatment	MI ± SE.	% abn. Mitoses ± S.E.	Prophase % abn.	Metaphase %abn.	Ana-telophase %abn.
Tomix tablets	2 tablets	24hr.	6.3 ± 1.00	29.07± 2.2	3.03	90.30	25.30
		48hr.	4.2 ± 0.12	34.78 ± 6.41	0.00	65.38	39.19
	3 tablets	24hr.	4.17 ± 1. 26	37.8 ± 1.9	3.51	83.30	30.40
		48hr.	8.03 ± 0.29	37.86 ± 2.1	10.84	76.74	23.60
Garlic powder	400mg	24hr.	9.63 ± 0.15	26.35 ± 1.5	4.80	77.40	8.49
		48hr.	4.3 ± 1.07	46.6 ± 7.1	12.82	87.50	51.78
	600mg	24hr.	3.6 ± 0.56	24.7 ± 3.7	12.00	72.70	30.60
		48hr.	6.3 ± 0.10	39.87 ± 4.3	17.24	64.28	75.68
Garlic fresh cloves	4.5gm/L	24hr.	10.23 ± 1.42	23.56± 0.6	7.04	43.90	43.28
		48hr.	5.84 ± 0.60	31.03± 2.5	6.30	68.80	40.90
	7.11gm/L	24hr.	2.03 ± 0.52	35.42± 2.2	18.18	60.00	53.57
		48hr.	5.1 ± 0.50	23.49 ± 1.8	5.41	62.50	28.78
control		24hr.	11.2 ± 1.64	6.18± 0.8	2.63	7.14	6.34
		48hr.	9.15± 1.7	8.26± 0.96	3.90	10.80	10.20

Table 2: Percentages of each abnormal type / scored mitosis number in *Vicia faba* root-tip meristems, after 24 &48hr of treatment with three different garlic forms.

Treatment	Concentration	Treat. time	% Different types of abnormal mitoses/scored number									
			Chromat. mater. Liquefac. abn.			Chromosomal kinetic abnormality					Chrom. structural aberr.		Micro-nu.
			Stic.	Sti.br.	Sum.	Dist.	Clump.	Lag.	Pro-meta	Sum.	Frag.&break.	Sum.	
Tomix tablets	2 tablets	24hr	16.36	0.00	16.36	6.66	0.00	0.00	6.84	13.5	0.60	0.60	0.00
		48hr	34.13	0.79	34.92	0.79	0.00	0.0	0.79	1.58	0.00	0.00	0.00
	3 tablets	24hr	10.90	0.68	11.58	24.65	0.00	3.4	3.42	31.5	0.68	0.68	0.00
		48hr	29.87	0.00	29.87	7.05	0.83	0.0	0.41	8.29	0.00	0.00	1.59
Garlic powder	400mg	24hr	15.90	0	15.90	0.43	0.69	2.4	0.86	4.38	0.00	0.00	2.40
		48hr	37.82	0.00	37.82	4.2	0.00	0.00	4.2	8.4	0.00	0.00	1.68
	600mg	24hr	18.10	0	18.10	5.17	0.86	0	0.86	6.89	0.00	0.00	0.86
		48hr	34.92	0.00	34.92	4.76	0.00	0.00	0.00	4.76	0.00	0.00	1.58
Garlic fresh cloves	4.5gm/L	24hr	13.71	0.88	14.59	8.40	0.88	1.3	0.88	10.58	0.00	0.00	1.30
		48hr	21.92	0.00	21.92	2.74	0.69	0.69	2.05	6.17	0.69	0.69	5.48
	7.11gm/L	24hr	27.86	3.2	31.06	6.50	0.00	0.0	0.00	6.50	0.00	0.00	0.00
		48hr	18.90	1.22	20.12	1.22	1.22	0.00	0.00	2.44	0.61	0.61	3.66
control		24hr	2.9	0.29	3.19	1.48	0.00	0.00	0.00	1.48	0.59	0.59	0.00
		48hr	4.36	0.00	4.36	2.54	0.363	0.00	0.00	2.90	0.00	0.00	0.36

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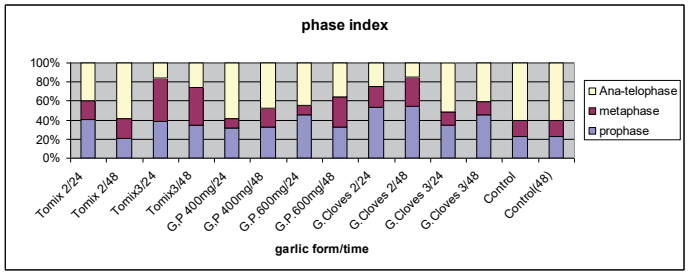

Figure 1: Phase index (PI) of Vicia faba root tip meristems after 24&28 hr treatment with the three different garlic products in different concentrations.

This was reversed after expanding the time of exposure to 48hr; 4.5gm/L of fresh cloves treatment had the highest effect on mitotic phases duration as it prolonged the prophase & metaphase frequencies by 54.11 & 30.82% on response of ana-telophase duration 15.06%. In the same track was the three tomix tablets induces the second highest effect on prophase and metaphase frequencies reaches 39.04, 45.21% on response of ana-telophase duration which was 29.87%.

24 hr of 400mg garlic powder affected the mitotic phases frequency but in different way of action, as it scored the highest Ana-telophase duration 66.23% (even if compared with control 60.9%) on response of metaphase duration 10.73%.

All concentration treatments of each Garlic forms inducing highly significant, increasing percentages of abnormal dividing cell of *Vicia faba* root meristems as shown in Table 3. The chromosomal aberrations were found to be concentration and time dependent most of the time except after treatment with 7.11gm/L of Garlic cloves for 48hr as it induced lower percentage than expected.

Worth to mention that although 24hr treatment with Garlic powder induce lower percentages of abnormal mitosis reaches 26.35% after 400mg and scored 24.7% after 600mg concentration, the 48hr treatment with 400mg of Garlic powder was the most destructive, since it induced the higher percentages of abnormal mitoses reaches 46.6 % followed by treatment with 600mg of Garlic powder form by 39.87%.

All studied Garlic forms induced chromosomes abnormalities which mainly are of chromatin material liquefaction type such as sticky chromosomes and sticky bridges as shown in Table 2 and Figured 2,4,7 and 8 followed by Chromosomal kinetic abnormality type such as pro-metaphase (Figures 3,4), disturbances in very low percentages (Figures 6,8) and clumped chromosomes (Figures 9) followed by chromosomal structural aberrations in a very few percentages (Figure 5).

After 24hr treatment; higher concentration of Garlic fresh gloves aqueous extract scored the higher effect of liquefaction on chromosome material by the time three tablets of tomix showed the least liquefaction effect on chromatin material. By expanding the time of treatment to 48hr, 400 mg followed by 600mg of Garlic powder showed the higher liquefaction effect on chromatin material by the time two tablets of tomix had the major kinetic effect.

Data revealed that 24 hr treatment with Garlic fresh raw & Garlic powder did not show any effect on chromosomes structure; despite of the low percentage of structural aberrations were scored after control (untreated faba plants) and after treatment with Tomix in its two concentrations. This was reversed after expanding the time of treatment to 48hr as the structure aberration was only noticed after treatment with Garlic fresh raw aqueous extract in its two concentrations. Regarding the micronucleus which comes as a result of structural aberration as real indicator for clastogenecity; data revealed that prolongation the time of treatment increase the risk of obtaining micronucleus; lower concentration of fresh garlic scored the higher clastogenecity 5.48% but this percentage was lowered by increasing the concentration to 3.66%.

On the bio-chemical investigation level, the total protein electrophoresis of treated *Vicia faba* seedling showed the effect of different garlic forms on the gene expression (Figure 10).

The resulted effects varied from appearance of some new bands to disappearance of other bands. The total number of bands in the control (untreated plants) was 24 bands ranging from 270 to 12 KDa. The lowest number of recorded bands was 21 after treatment with 7.11g/L of fresh cloves for 24hrs, while the highest bands number was 30 recorded after treatment with 7.11g/L of fresh cloves for 48hrs and treatment with two crushed tomix tablets for 24hrs. There were a thirteen common protein bands (monomorphic bands) showed at Mw 180, 130, 70, 51, 42, 40, 36, 27, 24, 22, 20, 19 and 15 KDa. Regarding the common effect within the two concentrations of each garlic form; protein analysis showed that: the 24hrs treatment with garlic fresh cloves recorded a common disappearance of protein bands at higher molecular weight 108, 65KDa. and a common appearance of new bands at lower molecular weight 23, 12 KDa, prolongation of treatment time to 48hrs treatment with fresh cloves showed protein band disappearance at 17KDa. and bands appearance at 28, 23 &12 KDa.

Treatment with crushed tomix tablets for 24 & 48hrs recorded a common disappearance of protein bands at 195, 95KDa and common appearance of new bands at 77, 28, 23 &12 KDa.

Treatment with garlic powder in the two concentrations for 24hrs recorded common band disappearance at 56 & 49 KDa and common band appearance at molecular weight 270, 150, 140, 47, 28 & 12 KDa. Prolongation the treatment time to 48hrs the common band disappearance recorded at molecular weight 195,108 KDa. and common band appearance recorded at molecular weight 28 & 12 KDa

In brief treatment with garlic products in each form stimulated some genes to be switch on and successfully translated into new protein, e.g. protein at Mw 12 KDa formed under the effect of different concentration of three garlic forms (fresh garlic cloves, garlic tablets and garlic powder) at 24 and 48 hours. The protein at Mw 23 KDa formed under the effect of different concentration of two garlic forms (fresh garlic cloves and garlic tablets) at 24 and 48 hours.

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Table 3: Effect of different garlic forms on protein electrophoresis patterns of *Vicia faba* (Giza 716) as plant model system.

No	MW	1	24 hour		48 hour		24 hour		48 hour		24 hour		48 hour	
			4.5g/L	7.11g/L	4.5g/L	7.11g/L	2 tab	3 tab	2 tab	3 tab	400 mg/L	600 mg/L	400 mg/L	600 mg/L
			2	3	4	5	6	7	8	9	10	11	12	13
1	270	-	-	-	-	-	-	-	-	-	+	+	-	-
2	195	+	+	+	+	+	-	-	-	-	+	+	-	-
3	180	+	+	+	+	+	+	+	+	+	+	+	+	+
5	150	-	-	-	-	-	-	-	-	-	+	+	-	-
6	140	-	-	-	-	-	-	-	-	-	+	+	-	-
7	130	+	+	+	+	+	+	+	+	+	+	+	+	+
8	108	+	-	-	+	+	+	+	+	-	+	+	-	-
9	95	+	+	+	+	-	-	-	-	-	+	+	-	+
10	90	-	-	-	-	+	+	-	-	-	-	-	+	-
11	85	+	+	-	+	+	+	+	+	+	+	+	-	+
12	77	-	-	-	-	+	+	+	+	+	-	-	-	+
13	70	+	+	+	+	+	+	+	+	+	+	+	+	+
14	65	+	-	-	+	+	+	+	+	+	+	+	+	-
15	56	+	+	+	+	+	+	+	+	+	-	-	-	+
16	51	+	+	+	+	+	+	+	+	+	+	+	+	+
17	49	+	-	+	+	+	+	+	+	+	-	-	-	+
18	47	-	-	-	-	-	-	-	-	-	+	+	+	-
19	44	-	-	-	-	-	+	-	-	-	+	-	-	-
20	42	+	+	+	+	+	+	+	+	+	+	+	+	+
21	40	+	+	+	+	+	+	+	+	+	+	+	+	+
22	36	++	++	++	++	++	++	++	++	++	++	++	++	++
23	32	-	-	-	-	+	+	+	-	-	-	-	-	-
24	30	+	+	+	+	+	+	+	-	+	-	+	+	+
25	28	-	-	-	+	+	+	+	+	+	+	+	+	+
26	27	+	+	+	+	+	+	+	+	+	+	+	+	+
27	26	-	-	-	-	+	+	+	-	-	-	-	-	-
28	25	-	-	-	-	-	-	+	+	+	-	-	-	-
29	24	+	+	+	+	+	+	+	+	+	+	+	+	+
30	23	-	+	+	+	+	+	+	+	+	-	-	-	-
31	22	+	+	+	+	+	+	+	+	+	+	+	+	+
32	21	+	+	-	+	+	+	+	+	+	+	-	+	-
33	20	+	+	+	+	+	+	+	+	+	+	+	+	+
34	19	+	+	+	+	+	+	+	+	+	+	+	+	+
35	18	+	+	-	+	+	+	+	+	+	+	+	+	-
36	17	+	+	+	-	-	-	+	+	+	+	-	+	-
37	16	-	-	-	-	+	+	-	-	-	-	-	-	+
38	15	+	+	+	+	+	+	+	+	+	+	+	+	+
39	12	-	+	+	+	+	+	+	+	+	+	+	+	+
Total		24	23	21	26	30	30	29	26	26	28	26	22	22

Discussion

Regarding the mitotic index as a parameter for the cytotoxicity and used as an indicators of adequate cell proliferation [18] and the percentage of abnormal mitosis as a parameter for the genotoxic effects of the tested materials; the cytogenetic investigations of 2500-3000 cells from three replicates were carried out in order to configure the real effects of the garlic plant product in its three tested forms.

Cytogenetic investigations show that, the reduction in the mitotic index after treatments were concentration and time dependent and reflect the cytotoxic effect of the garlic fresh cloves followed by the garlic powder then tomix tablets came the last; as it show the least reduction in the mitotic index. The slight improvement in the mitotic indexes recorded after 48h treatment with the higher concentration of Garlic in the tested forms (3cruched Tomix tablets, 600mg Garlic powder) reveals that the investigated plants under may have adapted the treatment or the 48hr aged Garlic product have lost some of its volatile components and did not show its assumed effect as 24hr aged Garlic form did (Table 2).

Concentration-dependent cytotoxicity of garlic product on plant cell was previously obtained by Sadaqa, et al. [19] when investigate the cytological effects of garlic aqueous extract on root tip cells of *A. cepa*. and also obtained by Suhasini, et al. [20] when tested on animal cells as it approved to reduce in mitotic index, induce growth arrest and morphological differentiation of MCF7 breast cancer cells.

The decrease mitotic index could be attributed to many reasons; it may be due to the alteration of DNA synthesis [21], blocking of the mitotic cycle during G2 [22], time consuming by repairing the damaged DNA at the check point after which the cell-cycle brakes are released and progress resumes [23], inhibition of protein synthesis [24] or the formation of various metabolites necessary for normal sequence of mitosis [25].

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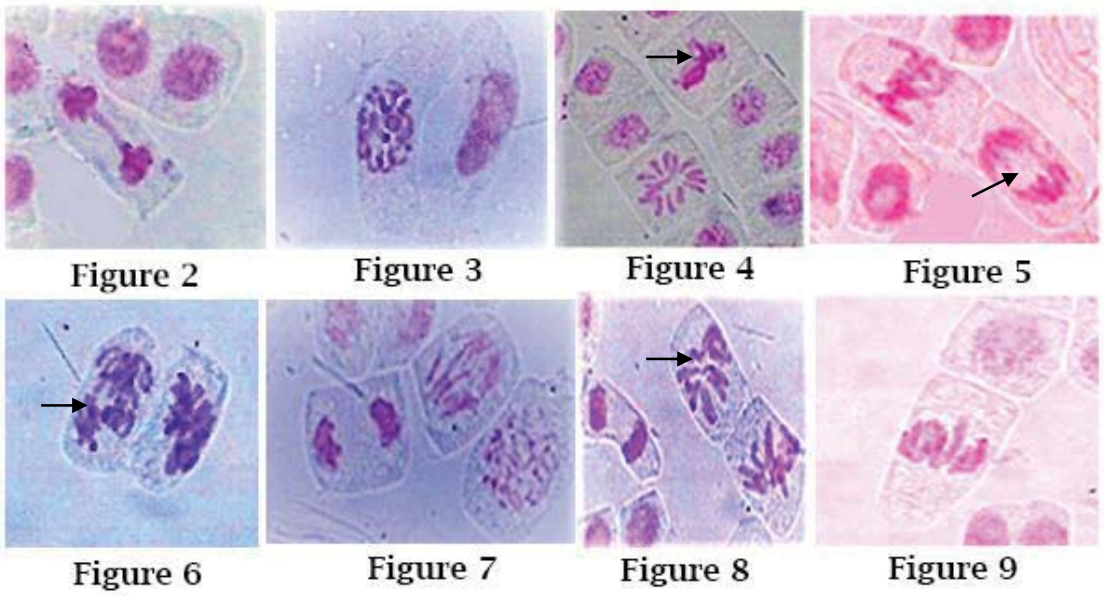

Figure 2-9: Sticky anaphase with sticky bridge after treatment with three mashed Garlic cloves past for 48hr (Figure 2), Pro-metaphase with ring chromosome after treatment with three mashed Garlic cloves past for 48hr (Figure 3), Sticky metaphase; C- metaphase after treatment with 400mg of Garlic powder foe 24hr (Figure 4), anaphase with broken structural bridge after treatment with three tomix tablets for 24hr (Figure 5), disturbed anaphase (arrow) & sticky metaphase after treatment with 600mg of garlic powder for 48hr (Figure 6) Sticky anaphase & disturbed anaphase after treatment with two tomix tablets for 48hr (Figure 7), sticky anaphase; disturbed metaphase (arrow) & sticky anaphase after treatment with two tablets of tomix powder for 24hr (Figure 8) and clumped metaphase after treatment with 600 mg og garlic powder for 24hr (Figure 9).

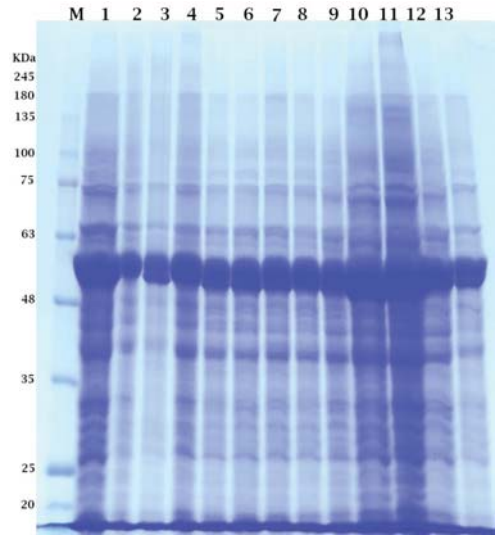

Figure 10: SDS-PAGE total proteins of Vicia faba seedlings pre-treated with different garlic forms.
M is the molecular weight protein marker.
1: Control. 2: 4.5g/L fresh garlic after 24 hrs. 3: 7.11g/L fresh garlic after 24 hrs. 4: 4.5g/L fresh garlic after 48 hrs. 5: 7.11g/L fresh garlic after 48 hrs. 6: 2 tabs of tomix after 24 hrs. 7: 3 tabs of tomix after 24 hrs. 8: 2 tabs of tomix after 48 hrs. 9: 3 tabs of tomix after 48 hrs. 10: 400 mg/L garlic powder after 24 hrs. 11: 600 mg/L garlic powder after 24 hrs. 12: 400 mg/L garlic powder after 48 hrs. 13: 600 mg/L garlic powder after 48 hrs.

This work also regarded the changes in duration of mitotic phases as an indicator for cytotoxic influence of the materials under investigations. Data revealed that garlic products affected notable change in the frequency of mitotic phases, mostly at prophase stage, as all forms prolong the prophase stages relative to control. Prolongation of prohases may be explained by that; the tested materials in some way block of the dividing cells prevent prophase-metaphase transition as a result of chromosomes condensation delay or the incomplete breakdown of the nuclear envelope or it may affects the chromosomes alignment on the equatorial plate or affect the spindle mechanism [26]. On the other hand treatment with three crushed tomix tablets induced the highest effect on metaphase frequencies, and 24 hr of 400mg garlic powder induced the highest effect on Ana-telophase duration, this prolongation of metaphases and ana-telophases may be explained by the difficulty in the separation of chromosomes into two chromatids, or could be resulted from the lengthening of their duration that leads to their accumulation ([27].

Regarding the chromosomal aberrations as an indicator to the mutagenic effect of the tested materials on chromosomes, all the concentration treatments in each of the Garlic forms induced, highly significant increasing percentages of abnormal dividing cell of *Vicia faba* root meristems. The chromosomal aberrations were found to be concentration and time dependent most of the time with only exception after treatment with 7.11g/liter of Garlic cloves aqueous extract for 48hr as it induced lower percentage than expected which may be explained by that Garlic in this form and concentration has increased the mitotic index by the way it could mask its mutagenic effect.

The cytotoxic effect of the 24hr treatment with three crushed tomix tablets followed by the 7.11g/liter of Garlic cloves aqueous extract was accompanied by the mutagenic effect as it produced the highest percentage of abnormal mitosis (37.8 &35.4%) mostly of chromatin material liquefaction, that was also empathized in case of three smashed garlic gloves by

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protein electrophoresis of treated plant leaves as it produces the lower number of recorded protein bands, three protein bands were completely disappeared which may be explained by that; the sever stickiness of chromatin prevent many genes from express themselves through transcription and translation into protein.

On the level of treatment for 48hrs; 400mg of Garlic powder scored the lowest mitotic index 4.3% and highest percentage of abnormal mitosis 46.6% mostly of chromatin liquefaction type, moreover it shows protein bands disappearance in proteins profile.

Regarding the structural aberration and micronucleus as an indicator for the clastogenecity, 24hrs treatment with Garlic fresh raw & Garlic powder did not show any effect on chromosomes structure despite the low percentage was scored in control and after treatment with Tomix tablets in its two concentrations; which may explained by that the chromotoxic effect of fresh cloves and garlic powder was sever as they prevent the fragments or breakage from complete separation from the chromosome or may explained by the corrective effect of these two form on the genetic material as Garlic described as an anticancer folk medicin [20]. Prolongation the time of treatment to 48hr; clarify the clastogenecty of the garlic fresh cloves. This clastogenecity effect was emphasized by the results obtained after the leaves' total protein electrophoresis as it was found 24hr treatment with Garlic fresh raw in its two concentrations and the Garlic powder 600 mg showed the higher number of disappeared bands, while 48hr treatment with Garlic powder in its two concentrations and tomix were the forms which showed the higher band disappearance.

Regarding the change in the protein profile as an indicator for the potential mutagenic effect of the tested materials on the plant cell: the appearance of new protein bands, the highest number bands recorded after; three smashed cloves for 48h and treatment with two crushed tomix tablets for 24h; this can be explained by mutational event at the regulatory system of unexpected gene(s) that activate it or synthesis of new protein controlled by function genes and /or it might be related to defense responses in the plants to protect themselves from harmful effect of the tested material [28-32].

Despite that treatment with the aqueous extract of three fresh cloves for 24hr showed mutagenic effect but not of clastogenic type, it showed the highly protein bands disappearance; so this disappearance can be attributed to the chromotoxic effect of the fresh cloves which prevent many genes from get expressed; rather than the clastogenic effect which reflect the genes loss via chromosomes breakage, fragmentation or laggard.

Conclusion

This study revealed that treatment with different concentration of garlic fresh cloves aqueous extract, and garlic powder in water for 24 & 48hr affected the mitotic activities of merestimatic cells. It also revealed that prolonging the time of treatment increases the risk of obtaining micronucleus, which is a countable indicator for the clasto-genecity of the tested product.

On the same track these products affected the total protein profile of *Vicia faba* seedling leaves. Therefore, it can be concluded that the usage of Garlic extracts as a raw fresh product or as powder in agriculture as fungi-pesticides, should be with caution, as they have been indicated to be mutagenic and cytotoxic on *Vicia faba* plant.

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