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Cytogenetic Effect of Allelochemicals *Brassica nigra* L. Extracts on *Pisum sativum* L.

Fawzia I. Mohamed and Zeinab M. El-Ashry

Department of Genetics and Cytology, National Research Centre, Dokki, Giza, Egypt

Abstract: Allelopathic potential of mustard extracts (*Brassica nigra* L.) was studied. Aqueous extract of mustard (*Brassica nigra* L.) at 0.25, 0.50 and 1% concentrations were applied to determine their effect on storage pea seeds germination, mitotic division, meiotic division and cell cycle. Increasing concentration of mustard extracts inhibited seeds germination. The data showed that mustard extract inhibited the cell division and increased the percentage of chromosomal aberrations in both mitotic and meiotic cell divisions of *Pisum sativum* plants. The lower percentage of mitotic index (MI) reached 7.44 and 7.99 % with 1% mustard extract after pea storage for 3&6 months compared to control (9.73 and 9.08%), respectively. Also, mustard extracts increased the percentage of chromosomal aberrations in mitoses which reached 14.92 and 12.94 % with higher concentration (1%) and storage for 3&6 months compared with control 0.98, 0.48%. The value of the meiotic abnormalities in pollen mother cells (PMCs) was 4.68 and 3.98 % after treated with 1% for storage 3&6 months as compared with the control 0.74 and 0.54 %. The types of abnormalities in both divisions were disturbed chromosome, stickiness, lagging chromosome and bridges. The cytomertic analysis revealed marked inhibition in the DNA synthesis in S phase and G_2 especially at treated with the highest concentrations of mustard extracts and storage for 3&6 months. Cytomertic measurement showed accumulation of cells in the G_0/G_1 phase and 4C-DNA. The percentages of cells having 2C-DNA content decreased while the cells having 4C-DNA increased.

Key words: Mustard extracts · *Pisum sativum* · Mitotic division · Meiotic division · Cell cycle

INTRODUCTION

Many plant species are still unevaluated chemically or biologically. Several studies regarding the action of plant extracts against some phytopathogenic fungi have been performed. The quality and quantity of the biologically active compounds from the plant extracts significantly depend on the species, the plant organ and harvest time [1, 2, 3, 4]. Brassica species is a wild plant, which are important oil seed crop and have potential for use as green manure crops [5]. Members of Brassicaceae have frequently been cited as allelopathic crop [6]. Allelopathy is an important interference mechanism where in one plant releases bioactive chemicals into the surrounding environment effect the growth of other plants [7]. It plays a significant role in agro- ecosystems and affects the growth, quality and quantity of product [8, 9]. Action of allelochemicals in target plant is diverse and affects a large number of biochemical reactions resulting in modifications of variety processes. The effects of allelochemicals action are detected at molecular, structural, biochemical, physiological and ecological levels of plant organization [10]. Jones [11] clearly defined the botanicals and plant allelochemicals as semi chemicals by organization for economic cooperation and development. The all chemicals involved in species communication (pheromones, plant extracts, plant volatiles and natural oils) and exhibiting pest control activities. Also, Gniazdowska and Bogatek [10] defined allelopathic as plant to plant, plant to microorganism and microorganism to microorganism interaction by chemicals belonging to the secondary metabolites (allelochemicals). Allelopathic compounds are released into the environment and usually provoke inhibitory effects in acceptor organisms.

Allelopathic compounds are chemical agents produced by a certain organism, to affect the health, growth, behavior, or population biology of organisms of

Corresponding Author: Fawzia I. Mohamed, Department of Genetics and Cytology, National Research Centre, Dokki, Giza, Egypt.

other species [12]. The vegetable extracts and their constituents can affect the life cycle of some insects and these same extracts can also interfere with the seed germination of some mono- and dicotyledonous [13, 14]. Allelopathy is one of the most important phenomenon influencing natural ecosystems, it may be also considered as a strategy of interference in competitive crop cultivars [15]. Many allelochemicals, produced by numerous species, are already known but in most cases, mechanism of their action remains unknown. Allelopathy is the search and development of new herbicides through the isolation, identification and synthesis of active compounds from allelopathic plants [16-19]. These compounds are often referred to as natural herbicides.

Physiological effects of allelochemicals include inhibition of photosynthesis and respiration, inhibition of enzymes activities, or modification in tissue water content. Little is known about cytological alterations induced by allelochemicals. There are only a few information describing modifications in morphology and structure of plant cells after treatment with allelochemicals. Allyl-isothiocyanates (ITC) isolated from black mustard (*Brassica nigra* L.) residues inhibited establishment of grass species. Other breakdown products of glucosinolate like ionic thiocyanate (SCN-) inhibited the root or shoot growth of many crop species [20]. It was also found in many studies that allelochemicals, which inhibited the growth of some species at certain concentrations, might stimulate the growth of same or different species at lower concentration [21]. The stimulatory (negative) allelopathic effects of any plant on the other plant can be used to develop ecofriendly, cheap and effective 'Green Growth Promoter's [22].

The present study was conducted to evaluate the cytogenetic activities of aqueous extract concentration of black mustard (*Brassica nigra* L.) in the root-tip cells and pollen grains of storage seeds of *Pisum sativum.*

MATERIALS AND METHODS

Black mustard (*Brassica nigra* L.) seeds were cleaned, air-dried and thoroughly ground to a fine powder. The seed powder (250 g) was first extracted with hexane (500 ml) to remove oil in Soxhlet apparatus for 110-115 h approx. The filtered seed meal was air dried for overnight and extracted with 500 ml of water by placing the mixture on shaker for about two nights. There after, the extract was filtered through a filter paper to remove particulate matter and the extract was dried by evaporating on water bath. Firstly the extract was solubilized in DMSO and then distilled water was added to make a total volume of 200 ml (stock). Fresh concentrations were prepared daily for each experiment. Then prepare different concentrations (0.25, 0.5 and 1%). *Pisum sativum* seeds, were weighting one Kg and divided into four groups. Three groups mixed with concentration of 0.25, 0.5 and 1 % mustard extracts. Fourth group, non-treated seeds used as control. The seeds aerated for 24 hrs at room temperature in order to dry and then stored for three and six months under normal conditions.

Seed germination: At the end of the storage periods, the seeds washed and soaked in tap water for 24hrs, then germinated in rolls of filter paper moistened with tap water. Three replicates were selected (15 seeds/replicate) for each treatment and the control. The percentage of seed- germination was estimated when the seedling was three days old:

$$\text{Relative germination Ratio (RGR)} = \frac{\text{Germination ratio of tested plant}}{\text{Germination ratio of control plant}} \text{x } 100$$

Percentage inhibition on germination of treatment plants to control were calculated, using the following formula as suggested by Sundra and Pote [23], Oyun [24]and Sazada *et al.* [25]:

$$I = 100 - (E2\ 100/E1)$$

where,
I = % inhibition,
E1 = Response of control plant,
E2 = Response of treatment plant.

Mitotic Studies: At the end of the storage period, the seeds washed and soaked in tap water for 24hrs, then germinated in rolls of filter paper moistened with tap water. The roots cut off when, 1.5-3.0 cm in length, fixed in acetic acid-ethyl alcohol (1:3) v/v and hydrolysis in 1N HCl for 10 min and stained using Feulgen squash technique. Three replicates were selected for each treatment and control and three roots were examined/ replicate. All experiments were conducted at room temperature (22±1ºC). The mitotic index and the mitotic inhibition were estimated as follows:

$$\text{Mitotic index} = \frac{\text{No. of total dividing cells}}{\text{No. of total counted cells}} \text{x } 100$$

$$\text{The mitotic inhibition} = \frac{\text{(Mitotic index in control- Mitotic index in treated)}}{\text{Mitotic index in control}} \text{x } 100$$

Chromosome abnormalities were scored in the pro- meta- and ana-telophase stage.

Meiotic Studies: At the end of the storage period, the treated seeds and control were soaked for 24 hrs in tap water and planted in pots with control. Flower buds were gathered. then fixed in Carnoy's and examined using the acetio-carmine smear method [26]. Three replicates were taken for each treatment and each replicate consisted of 3 plants. Abnormalities were counted the first and second meiotic divisions.

Cell Image Analysis: Fixed roots of *Vicia faba* seedling were stained by classical Feulgen stain. Image analysis on root cell nuclei was performed on Leica quantum 520 with a magnification power 40X. The organization, hard ware and soft ware package of this analysis have been described by Brugal [27]. The amount of DNA in the nucleus, DNA poloidy level and different phase of cell cycle were calculated. These include cells with DNA amount less than 2C value, cells with 2CDNA (G_0/G_1) cells with 3C-4C DNA (S phase) cells with 4C DNA (G_2 phase), cells with DNA amount more than the 4C value.

Statically Analysis: All cytological data were reported as mean values and Standard Error (SE) of the mean. SPSS computer software was used to estimate the t-test for significance at $p \leq 0.05$ and $p \leq 0.01$ level.

RESULTS AND DISCUSSION

Seed Germination: The results summaries the effect of mustard aqueous extracts on seed germination of *Pisum sativum* L. demonstrated in Table 1. The inhibitions of seed germination have been determined on aqueous extract concentration and storage period. In addition, it was determined that two storage time of *Pisum sativum* showed varied germination rate at similar concentration of aqueous extract. The relative germination ratio of seeds decreased with increasing concentration of extracts. The relative germination ratios of seeds are 48.42, 31.58 and 21.05 with 0.25, 0.50 and 1% of mustard as compared with control 100 after storage for three months. At further storage time for six months, the relative germination ratio of treated seeds decreases than three months. The percentages of inhibition on germination ratio reached to 20.83, 31.25 and 58.33% after the seeds storage for six months with 0.25, 0.50 and 1% of mustard extracts, while the percentage of inhibition on germination after storage three months were 51.80, 68.42 and 78.95% with the same concentrations. This means that the degree of inhibition on germination decreased as the decomposition periods progressively increased. On the other hand, the percentage of inhibition on germination was high percentage with high concentration 1% mustard extracts in the seeds storage for three months.

The toxic effects of aqueous extract of mustard on seed germination of *Pisium sativum* were evaluated. Data on the effects of the aqueous extract on seed germination of *Pisum sativum* showed in dose dependent manner. Inhibition of seed germination in *Pisum sativum* might be due to the present of allyl-isothiocyanates (ITC), glucosinolate like ionic thiocyanate (SCN-) and volatile compound. It might be also possible that the mechanism of action of the aqueous extract. Chang and Miller [28] support this similar study for black mustard extract on Lentil plant.

The results indicated that aqueous extracts of mustard at all concentrations markedly reduced the percentage of seed germination of pea seeds compared with the control. These results indicate that the inhibitory effect of the mustard extract on pea seeds germination agreed with several previous reports which noted that barnyard grass possessed stronger resistance against allelochemicals released by plants than did other weeds [29-32]. Obviously, the degree of inhibition increased as the concentration increased. Many studies suggested that the degree of inhibition increases with increasing extract concentrations [28, 32-35]. The reduction in phytotoxic activity of the allelochemicals over time is generally related to decomposition, moistly by soil microorganisms [36, 37].

Mitotic Studies: The differences in mitotic index values between control and treated *P. sativum* seeds with different concentration and storage time of mustard extract are presented in (Table 1). The mitotic index values in the control are similar after storage times. Effect on the mitotic index (MI) was decreased according to their treatment concentrations and time of storage. At the end of storage for three months, the lowest level of MI (7.44%) was observed with 1% mustard extract where MI was 9.73% in control. At the end of the six months storage period, the lowest level of MI (7.99%) was observed with 1% mustard extract where the control was 9.08%. With increasing the concentrations at the same storage period, there was statistically significant reduction of mitotic index values at ($P<0.01$). Highly statistically significant reduction of mitotic index values resulted at all concentrations after 3 & 6 months storage periods (Table 1). The degree of limit mitotic inhibition reached to 23.93% with 1% mustard extract and storage for 3 months. After storage for 6 months, the percentage of limit mitotic

Table 1: Relative germination ratio, Percentage inhibition of germination, mitotic index, percentage of limit mitotic inhibition and percentage of mitotic phases and percentage of abnormal phases in pea root-tip meristems after seeds treatment with different concentration of mustard extracts and storage for 3&6 months

						Mitotic phases					
						Prophase		Metaphase		Ana-telophase	
Time of storage (months)	Treatment %	Relative germination rate	% of Inhibition germination	% MI± SE	% limited of Mitotic inhibition	%	% abn	%	% abn	%	% abn
Three	Control	100	0.00	9.78±0.17	0.00	39.77	0.0	26.14	2.17	39.09	1.00
	0.25	48.42	51.80	8.39±0.31*	14.21	38.41	0.0	26.49	20.00	35.10	11.23
	0.50	31.58	68.42	8.29±0.21**	15.24	41.87	0.0	20.47	23.68	37.66	16.92
	1.00	21.05	78.95	7.44±0.21**	23.93	40.31	0.0	20.88	34.38	38.81	17.31
Six	Control	100	0.00	9.08±0.14	0.00	36.67	0.0	27.75	1.32	35.57	0.68
	0.25	79.17	20.83	8.61±0.16**	5.18	40.65	0.0	24.52	18.42	34.83	11.11
	0.50	68.75	31.25	8.33±0.05**	8.26	39.33	0.0	23.34	28.00	37.33	14.29
	1.00	41.67	58.33	7.99±0.31**	12.52	40.33	0.0	22.95	30.30	36.72	15.69

*Significant at level (P<0.05), **Significant at level (P<0.01)

inhibition slightly improved and reached to 12.52 %. This means that long periods of storage were improved the mitotic index.

From the results obtained the cytogenetic analysis indicated that mode of action for mustard extract toxicity involved disturbance of mitotic processes and induction of cell division aberrations. In the present study, the treatment of pea seeds induced a decrease in mitotic index (Table 1). The reduction in mitotic index suggests that exposure to mustard extracts led to cell cycle disturbances and decreases in cell number entering mitotic division. Due to the reduced number of dividing cells, mustard extract might have effects on cell division of pea and possibly be involved in blocking the DNA or protein synthesis required for normal cell division process. The inhibition of DNA or protein synthesis caused by mustard extracts may be the reason for the reduction in mitotic index.

The percentage of dividing cells in prophase, metaphase, anaphase and telophase was demonstrated in Table 1. For all treatment of concentrations and storage time, the metaphase stages decreased but prophase increase were observed. Data presented in Table 1 indicated that the percentage of abnormal phases, the abnormalities was noticed in metaphases, ana-telophases. The percentage of abnormalities in metaphase stage reached to 34.38% with 1.0% mustard extract after seeds storage for three months, while it reached to 30.30 % after seeds storage for six months with the same concentrations. The percentage of abnormal cells accumulated in metaphase stage. The percentage of abnormal cells in ana-telophases reached to 17.31 and 15.69% with treated seed of higher dose (1.0 %) mustard extracts and storage for three and six months, respectively. The mustard extract caused an increasing of aberration cells in root meristem cells in line with the increase of the concentration when compared to the control, yet it was also observed that these treatments caused statistically significant decreases in MI (Table 1).

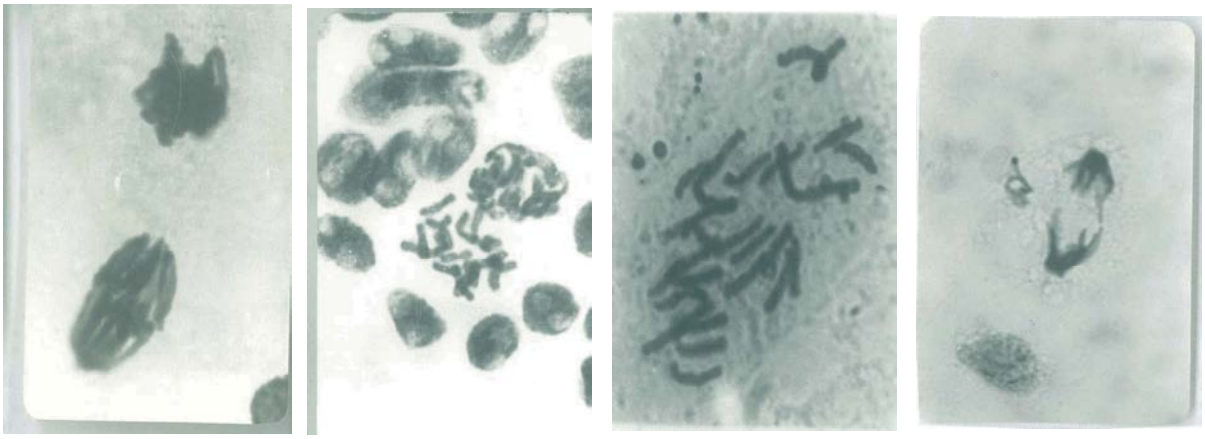
In all concentrations and periods of storage in the study, various abnormal cells were observed. The highly percentage of abnormalities reached to 14.92 and 12.99 % with high concentration 1% of mustard extract and seeds storage for three and six months compared to control 0.98 and 0.48 % respectively (Table 2). The observed anomalies in the study were: stickiness, disturbed, bridges, lagging and micro-nuclei (Table 2). Stickiness reached to 40.25% after treatment of pea seeds with 1% of mustard extract and storage for 6 months (Fig. 1a), Disturbed chromosome reached to 44.59 % after treatment with 1.0% of mustard extract and storage for three months (Fig. 1c, d), bridge occurrence in anaphase and telophase reached to 17.57 % after treated with 1.0 % of extracts and storage for 3 months and lagging chromosome are 7.69 % after the pea seeds treated with 0.5% and storage for 3 months (Fig. 1b).

These observations may be due to the nucleotoxic action of the extracts or the disturbance of the formation of spindle fibers during cell division, which leads to chromosomal aberrations. Stickiness and clumping of the chromosomes were some of the most common effects of these extracts on the root tips of pea after treated seeds and storage. Stickiness usually leads to the formation of anaphase and telophase bridges and this end up inhibiting post telophase, metaphase and cytokinins, respectively and thus hampering cell division. Stickiness might be due to the ability of the extracts to cause DNA depolymerization and partial dissolution of nucleoproteins, breakage and exchanges of the basic folded units of chromatids and the stripling of the protein

Table 2: Percentage of abnormal mitosis and the types of mitotic abnormalities in *P. sativum* root- tip meristems after –treatment with different concentrations of mustard extracts and storage for 3& 6 months

			Type of abnormalities relative to total abnormality			
Time of storage (months)	Treatment %	% of abn ±SE	Disturbed	Sticky	Bridge	Lagging
Three	Control	0.98±0.81	--	--	--	--
	0.25	9.48**±0.88	57.41	16.67	18.52	7.41
	0.50	12.06**± 0.50	44.59	29.23	18.46	7.69
	1.00	14.92**±0.58	--	32.43	17.57	5.41
Six	Control	0.48±0.22	--	--	--	--
	0.25	8.39**±0.38	39.89	39.78	15.76	4.57
	0.50	11.87**±0.18	40.02	38.44	17.09	4.45
	1.00	12.99**±0.59	39.73	40.25	14.53	5.49

**Significant at level (P<0.01)



A B C D

Fig. 1(a-d): Chromosomal abnormalities induced in root- tip meristems cells of *Pisium sativum* after seeds treatment with mustard extracts and storage. (a): Sticky metaphase and sticky anaphase after treatment with 1% mustard extracts for 6 months, (b): Disturbed metaphase after treatment with 0.5 %mustard extracts for 3 months, (c) Disturbed anaphase after treatment with 1% for, (d): Anaphase with lagging after treatment with 0.5% mustard extracts for 3 months

covering of DNA in chromosomes as also observed by Onyenwe [38]. These abnormalities have also been reported for several extracts and chemicals already investigated [39, 40, 41]. C-mitotic effects were observed with increase in concentrations of extract, Mitotic irregularities, such as incomplete anaphases or unequal distribution of chromosome to the daughter cells can result in aneuploid or even euploid cell. In Anaphase, when the two sets of chromosomes move to opposite poles, the section of chromatin between the centromere is stretched across between the poles, hindering separation into new daughter cells [42].

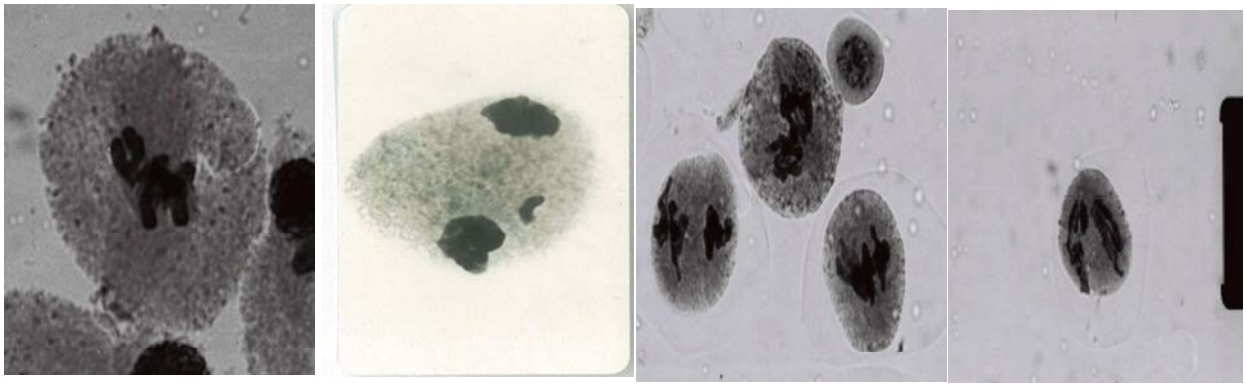
In general, numerical and structural changes in chromosomes are attributed to spindle failure leading to c-mitosis, nuclear fragmentation, lagging chromosomes, sticky chromosome and disturbed. Singh [43]. It is believed that cytotoxic extracts may lead to aneuploidy, doubled chromosome number, micronucleus formation or nuclear bud at the next stage of cell division [44]. The results showed that chromosome stickiness was one of the most frequent abnormalities induced by mustard extract in the root-meristem cells of pea. Chromosome stickiness causes extremely shortened and thickened chromosomes that are found in metaphase and anaphase. The presence of chromosome stickiness is an indication of mustard extract affecting organization of chromatin and suggests a possible role by which this cytotoxic agent may impact the physical and chemical properties of DNA, protein, or both, ultimately leading to improper folding of chromatin [45]. The clastogenic effects caused by the extracts included anaphase/telophase bridges, chromosome fragments and sticky chromosome. Babich *et al.* [46] reported that metaphases with sticky chromosomes lose their normal appearance and appear to

Table 3: Percentage of abnormal PMCs in meiotic division I and II in *P. sativum* plants and percentage of the different types of abnormalities in PMCs after treatment with different concentrations of mustard extracts and storage for 3&6 months

Time of storage (months)	Treatment %	No. of count PMCs ± SE	% abn of PMCs ± SE	% abn of PMCs ± SE in		Type of abnormalities relative to total abnormality			
				Meiotic division I	Meiotic division II	Disturbed	Sticky	Bridge	Lagging
Three	Control	4342	0.74± 0.11	0.59	0.87	--	--	--	--
	0.25	2690	1.99*± 0.31	2.06	1.83	24.53	50.94	18.87	5.66
	0.50	2653	2.74**±0.28	3.13	2.50	19.18	54.79	16.44	9.59
	1.00	2603	4.18**±0.31	5.75	2.85	23.85	50.46	18.34	7.35
Six	Control	3749	0.54±0.15	0.47	0.38	--	--	--	--
	0.25	1753	2.24**±0.78	3.62	1.58	28.21	56.41	12.82	2.56
	0.50	1652	3.73**±0.98	4.29	3.36	30.65	50.00	16.12	3.23
	1.00	1703	3.98**±0.85	5.69	2.80	30.88	48.53	16.18	4.41

* Significant at level (P<0.05)

**Significant at level (P<0.0



A B C D

Fig. 2 (a-d): Chromosomal aberrations in PMCs. of *Pisium sativum* plants after treatment with mustard extracts. (a)Sticky metaphase I after treatment with 1% for 3 months, (b) Anaphase I with lagging after treatment with 0.5 for 3months, (c) Sticky metaphase II after treatment with 1% for 6 months and (d) Anaphase II with bridge after treatment with 1% for 6 months

have a sticky "surface" which causes chromosome agglomeration, possibly due to effects on chromatin and chromosome organization. Singh [47] stated that the presence of chromosome fragments is an indication of chromosome breaks and can be a consequence of anaphase/telophase bridges.

Meiotic Studies: Seeds-treatment with mustard extract and storage for three and six months induced statically significant in the percentage of total chromosome abnormalities, first and second meiotic division and types of aberrations in the PMCs of *P. sativum* plant (Table 3). The percentage of abnormal PMCs in the first meiotic division was higher than that recorded in the meiotic division II after all treatments. The percentage of abnormal PMCs in first meiotic division reached to 5.75 and 5.69 % with 1%mustard extract after storage for three and six months compare to control 0.59 and 0.47 %, but second meiotic division reached to 2.85 and 2.80 % with the same concentration and storage time. The most common abnormalities were Stickiness, disturbed, anaphase and telophase bridges and lagging chromosomes (Table 3 and Fig. 2). The highest percentage of stickiness reached to 56.41% with 0.5% mustard extract after storage for six months. The percentage of disturbed chromosomes reached to 30.88 after treated with 1% mustard extract and storage for six months. The highest percentage of bridges reached to 18.87 with 0.5% mustard extract and storage for three months. Also, highly percentage of lagging chromosome found with 0.50 % mustard extract and storage for three months 9.59 % (Table 3).

All mitotic and meiotic irregularities were observed in these studies of the cytotoxic effects of mustard extract. Chromosome stickiness was the most famous type of

Table 4: Frequency of the cell cycle phases in the root meristem cells of *P. sativum* after seeds treatments with different concentrations of mustard extract and storage for 3 and 6 months

Time of storage (months)	Treatment %	DNA < 2C	G0/G1 phase	S- phase	G2 phase	DNA >2C
Three	Control	22.20	42.86	26.70	6.52	--
	0.25	20.09	48.93	21.81	2.50	2.63
	0.50	22.64	49.05	24.53	3.77	--
	1.00	21.81	56.00	18.69	4.66	3.33
Six	Control	26.20	40.57	28.60	7.10	--
	0.25	22.64	49.05	24.53	3.77	--
	0.50	22.92	47.91	22.91	5.00	1.25
	1.00	22.64	58.05	18.72	4.56	4.70

mitotic and meiotic irregularity that observed, which indicate to its highly toxic and irreversible effect which probably leads to cell death [48, 49]. The study indicated that the toxic effect of mustard extract considered as a result from chromatin irregularities that may lead to cell death. These results are similar to the result of two seeds extracts of *Sorghum bicolor* and *Nasturtium officinale* on *Vicia faba* plant [50].

Effect on Cell Cycle: Data in Table 4 indicated that the effect of mustard extract on the fraction of cell cycle phases of pea seeds. The most evident effect of this extract is the percentage of cells at the Go/G1 phases has increase with increasing concentration and time of storage. The percentage of cells in the DNA synthesis period (S-phase) decrease with the increase of concentrations and time of storage. Also, the percentage of cells in the G2-phase was decreased. The results obtained in this study cleared that the mustard extract act as inhibitor of cell cycle at S phases transition point and extended to G2 phase causing the recorded inhibition in the mitotic index. The variation in the nuclear DNA content related to the chromosomal aberrations was observed (lagging, disturbed type of spindle). This manifested by scoring of percentage of cells with DNA amount less the 2C value or more than the 4C value following treatment with mustard extract. The percentage of the cells 2C (G_o/G_1) were decrease than the control. The percentages reached 56.00 and 58.05 % after treatment with the highest concentration of mustard and storage for 3 and 6 months compared the control.

The percentage of cells 4C-DNA was increased by increasing the concentrations and the time of storage. Moreover, the percentage of S-phase progressively decreased with increasing of mustard extract concentrations and the time of storage. The percentage reached 18.72 % after treated with 1 % mustard extract and storage for 6 months compared with control (28.60 %). This result agree with that observed by Thomas *et al.* [51], Bruce *et al.* [52], Abo-El-Kier and Abd-El-Shafy [53], Lima *et al.* [54] and Abd-El-Hady and Barakat [55].

The present results indicated that mustard extract was reduced the number of nuclei having 3C-4C DNA and cells in G_2 phases. This means that mustard extract blocks the cells in G_1/G_2 phase causing inhibition of cell division (MI). This result is agreement with those reported by Polit *et al*. [56] who found that benzyl 6-aminopurine increased the number of G_2 cells and reported that there is a connection between phytohormones, gene expression and cell cycles in plants [57]. Badr *et al.* [58] indicated that the reduction of MI of *Vicia faba* roots by aflatoxin may be due to inhibition of DNA synthesis and also Kuras *et al.* [59] after treatment *Allium cepa* with bark water extract. The variation in the nuclear DNA content caused by mustard extract might be related to the chromosomal aberrations which observed (fragments, lagging and micro-nuclei in addition to disturbed type of spindle). This aberration may lead to an unequal distribution of genetic material in the daughter cells [60]. On the other hand, the fraction of cells in the DNA synthesis period (S phase and G_2 phase) and 3C and 4C-DNA decreased, where the replication of DNA and protein synthesis take place.

CONCLUSION

Our results indicate that mustard crude extract was inhibitor to germination. The experiments showed that reduction in the effect of mustard extract increased according to concentrations and period of storage. Significantly higher frequencies of cells with mitotic aberrations indicated the primary action of mustard extract to involve chromatin organization and mitotic spindles, leading to the induction of several abnormalities. Mustard extract affected on PMCs. of *P. sativum* plant after the seeds treated with 0.25-1% and storage for 3&6

months. The percentage of abnormal PMCs in the first meiotic division was higher than that recorded in the meiotic division II after all treatments. The various abnormal cells observed in mitotic and meiotic division. Aberrant cells observed according to concentration and time of storage of mustard extracts. The cytomertic analysis revealed marked inhibition in the DNA synthesis in S phase and G_2 especially at treated with the highest concentrations of mustard extracts and storage for 3&6 months. Cytomertic measurement showed accumulation of cells in the G_0/G_1 phase and 4C-DNA. The percentages of cells having 2C-DNA content decrease while the cells having 4C-DNA increased. These results indicated that the mustard extracts had a toxic effect on germination, mitotic, meiotic divisions and cell cycle. This means that using of mustard extract is not safe.

REFERENCES

1. Abu Bakar, E.L.M.M., 2009. Efficacy of crude extracts of garlic (*Allium sativum* Linn.) against nosocomial *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. J. Med. Plants Res., 3: 179-185.
2. Mahmoud, Abadi A.Z and M.K.G. Nasery, 2009. Antifungal activity of shallot, *Allium ascalonicum* Linn. (Liliaceae), *in vitro*. J. Med. Plants Res., 3(5): 450-453.
3. Pârvu, M., O. Roşca-Casian, M. Puşcaş and G. Groza, 2009. Antifungal activity of *Allium fistulosum* L. Contrib. Bot., 44: 125-129.
4. Pârvu, M, A.E. Pârvu, O. Roşca-Casian, L. Vlase and G. Groza, 2010. Antifungal activity of *Allium obliquum*. J. Med. Plants Res, 4: 138-141.
5. Grodzinsky, A.M., 1992. Allelopathic Effects of Cruciferous Plants in Crop Rotation. Chapter 6, Springers, Berline, pp: 77-85.
6. Bell, D.T. and C.H. Muller, 1973. Dominance of California annual grasslands by *Brassica nigra.* Am. Midl. Nat., 90: 277-299.
7. Rice, E.L., 1984. Allelopathy. 2nd Ed. Academic Press, New York.
8. Kohli, A., M. Leech, P. Vain, D.A. Laurie and P. Christou, 1998. Transgene organization in rice engineered through direct DNA transfer supports a two-phase integration mechanism mediated by the establishment of integration hot spots. Proc. Nat. Acad. Sci. USA, 95: 7203-7208.
9. Singh, H.P., D.R. Batish and R.K. Kohli, 2001. Allelopathy in agroecosystems: An overview. J. Crop Prod., 4: 1-41.
10. Gniazdowska, A. and R. Bogatek, 2005. Allelopathic interactions between plants. Multi site action of allelochemicals. Acta Physiol. Plant, 27: 395-407.
11. Jones, O.T., 2002. Commercialization of semiochemicals (an analysis of Potential barrier to the commercial development of semiochemicals for crop protection). Crop Protection Monthly, 148: 1-14.
12. Engel, S. and J.R. Pawlik, 2000. Allelopathic activities of sponge extracts. Mar Ecol-Prog Ser., 207: 273-281.
13. Dias, J.F.G., G.M. Círio, M.D. Miguel and O.G. Miguel, 2005. Contribuição ao estudo alelopático de *Maytenus ilicifolia* Mart. ex Reiss, Celastraceae. Rev Bras Farmacogn, 15: 220-223.
14. Fritz, D., A.P. Bernardi, J.S. Haas, B.M. Ascoli, S.A.L. Bordignon and G. Von Poser, 2007. Germination and growth inhibitory effects of *Hypericum myrianthum* and *H. polyanthemum* extracts on *Lactuca sativa* L. Rev. Bras Farmacogn, 17: 44-48.
15. Bhowmik, P. and C. Inderjit, 2003. Challenges and opportunities in implementing allelopathy for natural weed management. Crop Protect., 22: 661-671.
16. Duke, S.O., 1998. Potent phytotoxins from plants. In VII International Congress of Ecology 19-25 July 1998. (Ed. A. Farina, J. Kennedy and V. Bossù), pp: 120, Firenze, Italy.
17. Macias, D., Y. Gañan, T.K. Sampath, M.E. Piedra, M.A. Ros and J.M. Hurle, 1997. Role of BMP-2 and OP-1 (BMP-7) in programme cell death and skeletogenesis during chick limb development. Development, 124: 1109-1117.
18. Macias, F.A., J.M.G. Molinillo, R.M. Varela, A. Torres and R.O. Troncoso, 1998(a). Allelochemicals in sunflowers: Implications for crop and soil ecology management in agroecosystems. In VII International Congress of 19-25 July 1998. (Ed. A. Farina, J. Kennedy and V. Bossù), pp: 266, Firenze, Italy.
19. Macias, F.A., R.M. Oliva, A.M. Simonet and J.C.G. Galindo, 1998(b). What are allelochemicals? In Allelopathy in rice. Proceedings of the Workshop on Allelopathy in Rice, 25-27 Nov 1996. (Ed. M. Olofsdotter), pp: 69-79. IRRI Press, Manila.
20. Brown, P.D., M.J. Morra, J.P. McCaffrey, D.L. Auld and L. Williams, 1991. Allelochemicals produced during glucosinolate degradation in soil. J. Chem. Ecol., 17: 2021-2034.

21. Narwal, S.S., 1994. Allelopathy in crop production. Scientific Puplishers, Jodhpur, India. E.L. Rice, 1984. Allelopathy, 2nd Ed. Academic Press, New York. USA.
22. Oudhia, P., S.S. Kolhe and R.S. Tripathi, 1998. Allelopathic effect of *Blumea lacera* L. on rice and common Kharif Weeds. Oryza, 35: 175-177.
23. Sundra, M.P. and K.B. Pote, 1978. Allelopathic potentialities of root exudates from different ages of *Celosia argenta*. Natural Academic Science Journal, 1: 56-58.
24. Oyun, M.B., 2006. Allelopathic potentialities of *Gliricidia sepium* and Acacia auriculiformis on the germination and seedling vigour of maize (*Zea mays* L.). American Journal of Agriculture and Biological Sciences, 1(3): 44-47.
25. Sazada, S., Y. Ruchi, Y. Kavita, F.W. Ahmad, K.M. Mukesh, S. Sudarshana and J. Farah, 2009. Allelopathic potentialities of different concentrations of aqueous extract of some arable trees on germination and radicle growth of *Cicer arietinum* Var. C-235. Global Journal of Molecular Sciences, 4(2): 91-95.
26. Sharma, A. and K. Sharma, 1980. Chromosome Techniques: Theory and Practice. 3rd Ed., Butterworths, London, pp: 150-151.
27. Brugal, G., 1984. Image Analysis of Microscopic Preparation. In: Methods and Achievements in Experimental Pathology, Jasmin, G. and L. Proschek (Eds.). Karger, Basel, Switzerland, pp: 1-33.
28. Chung, I.M. and D.A. Miller, 1995. Natural herbicide potential of alfalfa residue on selected weed species. Agron. J., 87: 920-925.
29. Seal, A., T. Haig and J.E. Pratley, 2004. Evaluation of putative allelochemicals in rice root exudates for their role in the suppression of arrowhead root growth. J. Chem. Ecol., 30: 1663-1678.
30. Xuan, T.D., E. Tsuzuki, S. Tawata and T.D. Khanh, 2004. Methods to determine allelopathic potential of crop plants for weed control. Allelopathy J., 13: 149-164.
31. Khanh, T.D., I.M. Chung, T.D. Xuan and S. Tawata, 2005. The exploitation of crop allelopathy in sustainable agricultural production. J. Agron. Crop Sci., 191: 172-184.
32. Laosinwattana, C., W. Phuwiwat and P. Charoenying, 2007. Assessment of allelopathic potential of Vetivergrass (*Vetiveria* spp.) ecotypes. Allelopathy J., 19: 469-478.
33. Turk, M.A., K.D. Lee and A.M. Tawaha, 2005. Inhibitory effects of aqueous extracts of black mustard on germination and growth of radish. J. Agric. and Biol. Sci., 1(3): 227-231.
34. Laosinwattana, C., T. Poonpaiboonpipat, M. Teererak, W. Phuwiwat, T. Mongkolaussavaratana and P. Charoenying, 2009. Allelopathic potential of Chinese rice flower (*Aglaia odorata* Lour.) as organic herbicide. Allelopathy J., 24: 45-54.
35. Lawan, S.A., K. Suleiman and D.N. Iortsuun, 2011. Effects of allelochemicals of some Eucalyptus species on germination and radicle growth of *Arachis hypogea*. Bayero J. Pure and Applied Sciences, 4(1): 59-62.
36. Kobayashi, K., 2004. Factors affecting phytotoxin activity of allelochemicals in soil. Weed Biol. Manage., 4: 1-7.
37. Kobayashi, K., D. Itaya, P. Mahatamnuchoke and T. Pornprom, 2008. Allelopathic potential of itchgrass (*Rottboellia exaltata* L.) powder incorporated into soil. Weed Biol. Manage., 8: 64-68.
38. Onyenwe, C.N., 1983. Cytological effects of seed extracts of *Abrus procatorius* on the mitosis of *Allium cepa* and the effect of root extract of *Boerhaavia diffusa* on mitosis of *Crassocephallum biafrae*. University of Port Harcourt, Port Harcourt.
39. Badr, A. and T.T. Elkington, 1982. Antimitotic and chromotoxic activities of Isoproturon in *Allium cepa* and *Hordeum vulgare*. Environmental and Experimental Botany, 2: 265-270.
40. Misra, M.P., 1982. Effect of calcium salts on *Allium cepa* chromosomes. Cytologia, 47: 47-51.
41. Nwakanma, N.M.C., P.G.C. Odeigah and B.O. Oboh, 2009. Genotoxic effects of *Gongronema latifolium* and *Vernonia amygdalina* using the *Allium* test. In: Book of Proceedings, 4th UNILAG Conference and Fair, Nigeria, October 21-22, 2009, 81-90.
42. Bhujbal1, S.S., R.P. Deshmukh, J.S. Bidkar, V.A. Thatte, S.S. Awasare and P.P. Garg, 2011. Evaluation of cytotoxic activity of barks of *Mimusops elengi*. Eurasia J. Biosci., 5: 73-79.
43. Singh, I., 2000. Pidgins and Creoles: An Introduction, Oxford University Press.
44. Singh, R.J., 2002. Plant Cytogenetics. CRC Press, London.
45. El-Ghamery, A.A., M.A. El-Kholy and M.A. Abou El-Yousser, 2003. Evaluation of cytological effects of Zn2+ in relation to germination and root growth of *Nigella sativa* L. and *Triticum aestivum* L. Mutat. Res., 537: 29-41.

46. Babich H., M.A. Segall and K.D. Fox, 1997. The *Allium* test a simple, eukaryote genotoxicity assay. Am. Biol. Teach., 59: 580-583.
47. Singh, R.J., 2003. Plant cytogenetics. CRC Press, Boca Raton, pp: 463.
48. Fiskesjo, G., 1988. The Allium test-an alternative in environmental studies: the relative toxicity of metal ions. Mutat. Res., 197: 243-260.
49. Fiskesjo, G., 1997. *Allium* test for screening chemicals, evaluation of cytological parameters. In: W. Wang, J.W. Gorsuch and J.S. Hughes,(Eds.), Plants for Environmental Studies. CRC, Lewis Publishers, New York, pp: 307-333.
50. Adam, Z.M., E. Mikhael, Z.M. El-Ashry, N.O. Ehsan and R.T. Ali, 2008. Cytogenetic and Biochemical studies on *Vicia faba* plants treated with two insecticidal active plant extracts. J. Genet. Engin. And Biotech., 6(2): 19-27.
51. Thomas, M., V. Von Hagen, Y. Moustafa, M.P. Montmasson and J.D. Mont, 1991. Effect of gossypol on the cell cycle phases in T-27D human breast cancer cells. Anticancer Res., 11: 1469-1476.
52. Bruce, A., B. Dennis, L. Julian, R. Martin, R. Keith and D. James, 1994. Molecular Biology of Cell. 3rd Ed., Garland Publishing Inc., New York, USA., ISBN-13: 978-0815316190, Pages: 1408
53. Abo-El-Kier, Z. and E. Abd-El-Shafy, 1998. The response of *Vicia faba* to high concentrations of Cadmium. Egypt. J. Genet. Cytol., 27: 205-217.
54. Lima, De P.D., P.C. Cardoso, A.S. Khayat, O.B. Med and R.R. Burbano, 2003. Evaluation of the mutagenic activity of hydroxyurea on the G_1-S-G_2 Phases of cell cycle: an *in vivo* study. Genet. Mol. Res., 2: 328-333.
55. Abd-El-Hady, A.E.A. and H.M. Barakat, 2005. Effects of four food preservative chemicals on chromosomal aberration, gene expression and DNA image dueing cell cycle in *Allium cepa* and *Vicia faba* plants. Egypt. J. Genet., Cytol., 34: 237-254.
56. Polit, J.T., J. Maszewski and A. Kazmierczak, 2003. Effect of BAP and IAA on the expression of G1 and G2 control points and G1-S and G2-M transitions in root meristem cells of *Vicia faba*. Cell Biol. Int., 27: 559-566.
57. Mohanty, S., A.B. Das, P. Das and P. Mohanty, 2004. Effect of a low dose of Aluminum on mitotic and meiotic activity, 4C DNA content and pollen sterility in rice, *Oryza sativa* L. Ecotoxicol. Environ. Saf., 59: 70-75.
58. Badr, A., A.S. Shehab, Z.H. Kheiralla and H.H. El-Shazly, 1995. Mutagenic potential of aflatoxins produced by *Aspergillus parasiticus* and its effect on growth and yield of *Vicia faba*. Proceedings of the 3rd Cong. Toxicol. Dev. Count. Vol. 2, November 19-23, Cairo, pp: 99-114.
59. Kuras, M., J. Nowakowska, E. Sliwinska, R. Pilarski and R. Iiasz, 2006. Changes in chromosome structure, mitotic activity and nuclear DNA content from cells of Allium test induced by bark water extract of *Uncaria tomentosa* (Willd) DC. J. Ethnpharmacology, 17(2): 211-221.
60. Gavrila, L., A. Lungeanu, I. Rebedea, L. Ghetea, V.R. Gavrila and L.B. Gavrila, 1994. The study of the mutagenic effects of zinc phosphide a raticide and of Mitomycin C (an alkylating agent) in presence of activated waters. Cytologia, 59: 81-86.