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Determination of the genotoxic effects of *Trigonella foenum graecum* L. extracts in stored *Pisum sativum* seeds

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Abstract: This study was carried out to study the effect of *Trigonella foenum graecum* L extracts on mitotic, meiotic and cell cycle parameters in stored *Pisum sativum* seeds. The *Trigonella foenum* extracts induced a marked decrease in the mitotic index accompanied with considerable percentage of chromosomal aberrations. These abnormalities include stickiness, disturbed chromosomes, bridges and lagging chromosome. Cytophotometric measurements showed inhibition of cells in the G_0/G_1 phase at the expense of other phases of cell cycle (S-phase, G_2-phase). The percentage of cells having 2C DNA content decreased gradually with increasing the concentrations, while those having 4C DNA increased. *Trigonella foenum* extracts not affected on PMCs. Of *P. sativum* plant after the seeds treated with 0.5-1% and storage for three months but, significant percentage of chromosome abnormalities observed in the PMCs of the plants treated with 2% *Trigonella foenum* extracts and storage for three months. Also, after storage for six months all treatments have significant effect on abnormalities of PMCs. In all the treatment, various abnormal cells were observed in mitotic and meiotic division. Aberrant cells observed according to concentration and time of storage of *Trigonella foenum* extracts.

Keywords: Cell cycle, meiotic division, mitotic division, *Pisum sativum* seeds, *Trigonella foenum graecum* L. extracts

INTRODUCTION

Botanical insecticides are naturally occurring insect toxins extracted from plants. Several such chemicals have been formulated for insect management in the home garden. They act quickly, causing immediate paralysis, death, or cessation of feeding. Many botanical insecticides, but not all, are less toxic than synthetic pesticides to mammals and plants. Botanical insecticides also tend to break down rapidly in the environment.

Fenugreek is a member of the Leguminasae (Fabaceae) family and is commonly cultivated in India, Egypt, the Middle East and North Africa. The seeds of the plant have been used as a traditional remedy for numerous conditions including gastrointestinal disorders, gout, wound healing and inflammation, hyperlipidemia and diabetes.

Fetrow and Avila (1999) found that bioactive compounds isolated from fenugreek seeds include saponins, alkaloids, amino acids, flavinoids, some of which act as insulin secretogogues, coumarins, mucilaginous fibers, nicotinic acid and other vitamins and minerals. Flavonoids have remarkable biological activities, including inhibitory effects on enzymes, modulatory effect on some cell types, protection against allergies, antibacterial, antifungal, antiviral, anti-malarial, antioxidant, anti-inflammatory and anticarcinogenic properties Donatus *et al*. (2011).

Plant extracts and their essential oils are one of several non-synthetic chemical control options that have recently received attention for controlling plant diseases (Khater, 2003; Soylu *et al.*, 2005; Abad *et al*., 2007). The methanolic extract of fenugreek was potent in inhibiting dermatophytes and *Candida albicans* (Shtayeh and Abu Ghdeib, 1999; Olli and Kirti, 2006). Extracts of harmal seeds and roots were found to contain a mixture of active alkaloids and among these harmaline was the most active antifungal agent (Telezhenetskaya and D'yakonov, 2004).

Very little information is available on the use of plant extracts on the insect pests of stored grains, especially on the *p. sativum*. There fore the study was inhibited to find and recommend possibly the most effective plant extract against *S. oryzae* in the stored. Highly evolved plants are an important source of novel insecticides (Prakash and Rao, 1997). Numerous plant species or extracts from plants have insecticidal properties, this mean that, the action of vegetable oils is due to suffocation oils can act as antifeedants or even act as Insect Growth Regulators (IRGs) by affecting metamorphosis. Jacobson (1989 and Golob *et al*. (1999).

Weaver and Subramanyam (2000) suggested that fumigant activity in botanicals could have a greater potential use than grain protectants in future based on their efficacy, economic value and use in large-scale storages.

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The present investigations were under taken to study the cytogenetic activities of fenugreek extract (*Trigonella foenum graecum* L.) in the root-tip cells and pollen grains of stored seeds of *P. sativum.*

MATERIALS AND METHODS

This study was conducted at Laboratory of genetic and cytology in the National Research Center in Egypt, in 2011.

Seeds of *Pisum sativum* (V. Mater B1) and *Trigonella foenum graecum* L seeds were used.

Many *Pisum sativum* seeds, weighting 1 Kg, mixed with concentration of 0.5, 1. and 2% fenugreek extracts. The seeds aerated for 24 h at room temperature in order to dry and then stored for 3 and 6 months under normal conditions. Non-treated seeds used as control.

Preparation of seeds extract: The extract was prepared by boiling 4 g of seeds of fenugreek (*Trigonella foenum graecum* L) mixed with 200 mL distilled water for covered beaker (2% stock solution) for 5 min and, cooled to room temperature for 10 min. Also, prepare 2 g/200 mL (1%) and 0.5 g/100 mL (0.5%).

Seed germination: At the end of the storage periods, the seeds washed and were soaked in tap water for 24 h, 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 were three days old.

Mitotic studies: At the end of the storage period, the seeds washed and soaked in tap water for 24 h, 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 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:

mitotic index = No. of dividing cells/No. of dividing cells + No. of interphase cells X 100. The mitotic inhibition = (mitotic index in control-mitotic index in treated) X 100/mitotic index in control. Chromosome abnormalities were scored in the pro-meta-and ana-telophase stage.

Cell image analysis: For cytometric measurement of nuclear DNA content, at least 400 cells sampled for each treatment were to be used. Feulgen-stained slides were scanned according to Fukui (1988) using cell image processing system (Computerized Image Analysis in Scientific Applications System-Quantiment 520 Leica). The amount of DNA in the nucleus, DNA ploidy level and different phases of cell cycle were calculated. These induced; cells with DNA amount less than the 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 4C value.

Meiotic studies: At the end of the storage period, the treated seeds and control were soaked for 12 hrs in tap water and planted in pots with control. Flower buds were gathered. Its fixed in Carnoy's and examined using the acetio-carmine smear method (Sharma and Sharma, 1980). Three replicates were taken for each treatment and each replicate consisted of 3 plants. Abnormalities were counted the first and second meiotic divisions.

The data were statistically analyzed using one-way Analysis of Variance (ANOVA).

RESULTS

Table 1 clear that the percentage of seeds germination of *P. sativum* after treatment with fenugreek extracts and storage for three and six months were lower than control. The percentage of germination revealed a significance decrease and reached to less half with treatment 2% fenugreek extracts and storage for 3 months compared to control. The percentage of germination improved after long time of storage.

The mitotic activities and the chromosomal aberrations observed in the cells of the treated storage *P. sativum* seeds and control shown in Table 1 and 2. The mitotic index values estimated for *P. sativum* root cells found to be lower with the higher concentrations after storage seeds for 3 and 6 months compared to the control root cells. The lowest mitotic index value of 8.63% was recorded for treated with 2% fenugreek extract and storage for 6 months. The values of mitotic inhibition were lower in 6 months than the 3 months.

As regards the effect of fenugreek extract on the percentage of different mitotic phases, the fenugreek extract have low decrease effect on metaphase stage (Table 1). No abnormalities were observed in the prophase stage. The percentage of abnormalities was higher in *P. sativum* root-tip meristems after seeds-storage for all concentrations and time of storage. The highest percentage of chromosomal abnormalities was 4.58±0.04 ($p<0.01$) in the root-tip meristems of *P. sativum* seeds treated with 2% and storage for 6 months. Prolongation of the storage period to 6 months had no lowering effect on the percentage of the induced abnormalities in the root-tip cells of *P. sativum* (Table 2).

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

					Mitotic phases					
					Prophase		Metaphase		Ana-telophase	
Treatment	Storage periods (months)	% of germination	% of MI±S.E.	% limited of mitotic inhibition	%	% abn.	%	% abn.	%	% abn
Control	three	93.33	10.72±0.27	0.00	38.83	0.0	27.74	3.0	33.43	2.49
0.5		66.67	9.73±0.35	9.24	38.49	0.0	26.74	10.40	31.07	2.98
1.0		43.00	9.43±0.13	12.03	38.40	0.0	25.60	7.50	36.00	4.44
2.0		36.67	8.88**±0.17	17.16	37.95	0.0	23.76	13.69	33.68	4.06
Control	six	96.67	9.52±0.12	0.00	38.83	0.0	27.42	3.47	33.76	2.35
0.5		83.33	9.33±0.13	1.99	40.45	0.0	24.27	4.67	35.28	2.40
1.0		73.33	9.06±0.14	4.62	39.46	0.0	23.41	12.14	33.44	2.50
2.0		70.00	8.63*±0.16	9.35	40.56	0.0	22.93	7.69	36.51	7.73

*: Significant at level (p<0.05); **: Significant at level (p<0.01)

Table 2: Percentage of abnormal mitoses and the types of mitotic abnormalities in *P. sativum* root-tip meristems after-treatment of seeds with different concentration fenugreek extracts and storage for 3 & 6months

			% of the different type of abnormalities relative to total abnormality			
Treatment	Storage periods (months)	% of abn.±S	Disturbed	Sticky	Bridge	Lagging
Control	three	1.75±0.71				
0.5		0.65±3.74 *	45.17	37.17	12.67	4.49
1.0		±0.45 3.53*	36.36	36.36	18.18	9.09
2.0		4.64**±0.58	40.74	33.33	22.22	3.70
Control	six	0.54±0.22				
0.5		2.18**±0.95	34.79	44.79	16.69	3.73
1.0		3.92**±0.31	36.89	49.06	10.21	3.84
2.0		4.58**±0.04	37.53	48.82	12.71	2.94

*: Significant at level (p<0.05); **: Significant at level (p<0.01)

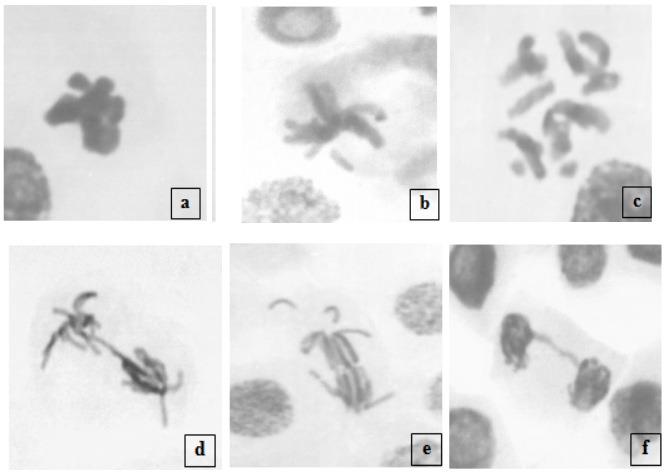

Fig. 1: Chromosomal abnormalities induced in root-tip c ells following treatment of pea seeds with fenugreek extracts and storage for 3&6 months. Sticky metaphase, (a), lagging metaphase, (b) treated with 1% fenugreek extracts and disturbed metaphase, (c) with 0.5% fenugreek extracts and storage for 3 months, anaphase and telophase with bridge (d and f) and lagging anaphase, (e) with 2% fenugreek extracts in root-tip meristems of pea seeds

Aberrant cells observed according to concentration and time of storage of the extract, in all the treatment. Anomalies that were observed in this study are as follows: Stickiness reached to 49.06% after treatment of pea seeds with 1% of fenugreek extract and storage for 6 months (Fig. 1a), Disturbed chromosome reached to 45.17% after treatment with 0.5% of fenugreek extract and storage for three months (Fig. 1c), bridge occurrence in anaphase and telophase reached to 22.22% after treated with 2% of extracts and storage for 3 months (Fig. 1d and f) and lagging chromosome are 9.09% after the pea seeds treated with 1% and storage for 3 months (Fig. 1b and e) (Table 2).

The statistical analysis on the relationship of the different concentrations of fenugreek extracts with mitotic index and chromosomal aberration data revealed a strong

Table 3: Effects of fenugreek extracts on the measured cell cycle parameters in root meristematic cells of *P. sativum* seeds after treatmrnts and storage for 3 &6 months

Time of storage	Concentration g/L	DNA<2C	G_0/G_1 phase	S-phase	G_2 phase	DNA>4C
3	control	12.28	79.83	7.89	00.00	00.00
	0.5	18.63	68.62	11.77	0.98	00.0
	1.0	5.56	51.85	26.85	12.96	2.78
	2.0	4.43	43.36	37.17	11.50	3.54
6	control	10.00	78.50	11.50	00.00	00.00
	0.5	8.78	56.53	14.17	15.83	4.69
	1.0	8.93	66.07	22.00	3.00	00.00
	2.0	7.14	58.16	26.53	8.17	00.00

Table 4: 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 treatments with fenugreek extracts different concentration and storage for 3 & 6 months

				% of abn. PMCs in		% of type abnormalities relative to the number of abnormal meioses			
Treatment	Time of storage (Month)	No of count cells PMCs	% abn of PMCs ±S.E.	MDI	MDII	Stick	Dist.	Bridg.	Lagg.
Control	three	6243	0.45 0.03	0.25	0.82	----	---	----	----
0.5		2684	0.94 0.09	0.97	0.90	40.00	32.00	20.00	8.00
1.0		2790	1.08 0.28	1.42	0.76	56.67	20.00	13.33	6.67
2.0		2615	1.73*±0.56	1.97	1.56	66.67	15.56	11.11	6.67
Control	six	2568	0.62±0.15	0.65	0.59	-----	-----	----	-----
0.5		1985	1.76*±0.78	2.34	1.33	62.86	22.86	11.43	2.86
0.1		1763	2.84**±0.98	4.44	1.67	48.07	37.93	11.14	2.86
2.0		1533	3.33**±0.85	4.43	2.40	45.47	41.35	9.34	3.84

*: Significant at level (p<0.05);:** Significant at level p<0.01)

positive relationship (Table 1 and 2). The analysis showed significant relationship between the treatments and the different values of mitotic index and chromosomal aberration percentages.

The effect of fenugreek extracts on the fraction of cell cycle phases is clear in Table 3.

The most evident effect of this extract is the percentage of cells at the G_0/G_1 phases has decrease with increasing concentration and time of storage. The percentage of cells in the DNA synthesis period (S-phase) increased with the increase of concentrations and time of storage. Likewise, the percentage of cells in the G_2-phase was increased. The results obtained in this study, clear that the fenugreek extract act as inhibitor of cell cycle at G_0/G_1 transition point and extended to G_2 phase causing the recorded inhibition in the mitotic index.

The variation in the nuclear DNA content related to the chromosomal aberrations 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 fenugreek extract.

Seed-treatment with 0.5-1.% fenugreek extracts and storage for 3 months had no effect on meioses but with 2% fenugreek extracts give statically significant in the percentage of chromosome abnormalities in the PMCs of *P. sativum* plant. All treatments and storage for 6 months have statically significant in the percentage of chromosome abnormalities in the PMCs Table 4.

The percentage of abnormal PMCs in the first meiotic division was higher than that recorded in the meiotic division II after all treatments. The most common

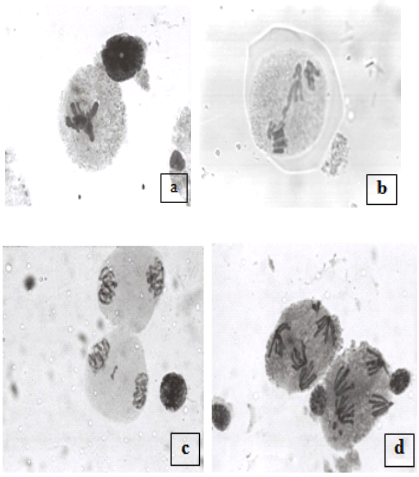

Fig. 2: Sticky metaphase I, (a) and anaphaseI with bridge from PMCs of *Pisum sativum* plants after treated seeds with 2% fenugreek extracts and storage for 3 months, (b). Anaphase I with lagging chromosome, (c) and anaphase II with lagging, (d) after treated with 1% fenugreek extracts and storage for 6 months

abnormalities were Stickiness, disturbed, anaphase and telophase bridges and lagging chromosomes (Table 4 and Fig. 2a-d).

DISCUSSION

From our results, the treatment seeds of *P. sativum* with fenugreek extracts affect on the percentage of

germination after 3 and 6 months storage. The percentage of germination improved after storage for 6 months. This finding was agree with that previously studies by Abou elkeir and Abou elkeir (1992), Haroun and Al shehri (2001), Sunar *et al*. (2009), Laosinwattana *et al*. (2009) and Haroun (2010).

The lower value in mitotic index in the root-tip cells after treatment of *P. sativum* seeds with fenugreek extracts and storage for 3 and 6 months indicates inhibition of cell division than the value of control. This suggests the suppression of mitotic activities in *P. sativum* seeds treated with fenugreek extracts and storage, since mitotic index is a quantitative estimation of the mitotic activities in an organisms or a particular organ of an organism. This observation corroborates the findings of Bakare *et al*. (2000), who recorded lower mitotic index values in the treated root cells of *A. cepa* when compared with the control root cells.

In the light of the results obtained in the present study, these observations above may be due to the nucleotoxic action of the extracts or disturbance of formation of spindle fibers during cell division which leads to chromosomal aberrations. Stickiness of chromosomes were the most common effects of these extracts on the root tips cells and PMCs. These abnormalities have also been reported for several extracts and chemicals already investigated (Badr and Elkington, 1982; Nwakanma *et al*., 2009; Nwakanma and Okoli, 2010). Stickiness usually leads to the formation of anaphase and telophase bridges and this end up inhibiting metaphase and cytokinesis, 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 covering of DNA in chromosomes as also observed by (Onyenwe, 1983; El-Ghamery *et al*., 2003).

Disturbed meta-and ana-telophases were the major types of abnormalities observed in somatic and pollen mother cellsof *P. sativum* plant after treatment with the fenugreek extracts and storage. El-Ghamery *et al*. (2003) suggested that the Spanish jasmine extract may be impacting the spindle apparatus in root tip cells of *A. cepa* L.

Lagging chromosome and ana-telophase bridges were observed in the mitosis and meioses Teerarak *et al*. (2010) reported that *Jasminum offcinale* L. extracts induce chromosomal aberrations in *Allium cepa* roots

The chromosomal aberrations in the root-tip cells were definitely induce by the ingredients contained in the fenugreek extract since such aberrations did not observed in the control. This suggests that the fenugreek extract could be genotoxic at the chromosomal level, especially at high concentrations. The chromosomal aberrations are parts of the hazards associated with herbal medications due to effects of the chemicals such as alkaloids, flavonoids, terpenoids, tannis, carcinogens, contained in plants (Kurnkum, 1993).

The observation of laggard, anaphase bridge and scattering of chromosomes at anaphase is an indication of the capability of fenugreek extract in causing chromosome breakage, resulting in genetic imbalance in the genome.

The present data showed that fenugreek extracts elevated the number of S phase and G_2 cells but lowering G_o/G_1 phase cells. This result agree with that of Kaviarasan *et al*. (2006) suggested that the polyphenolic compounds of fenugreek seeds can be decrease the accumulation of sub-G_1 phase cells. The fenugreek extracts prevent ethanol-induced toxicity and apoptosis in change liver cells.

Treatment of *P. sativum* with fenugreek extracts induced a significant percentage of abnormal PMCs. This results similar result of two seeds extracts of *Sorgum bicolor* and *Nasturtium officinale* on *Vicia faba* plant (Adam *et al*., 2008).

CONCLUSION

These results cleared that the percentage of germination reached to less half with treatment high concentration 2% fenugreek extracts and storage for three months compared to control. The percentage of germination improved after storage for 6 months. The *Trigonella foenum* extracts induced a marked decrease in the mitotic index accompanied with considerable percentage of chromosomal aberrations. Also, *Trigonella foenum* extracts inhibition of cells in the G_o/G_1 phase at the expense of other phases of cell cycle (S-phase, G_2-phase). The percentage of cells having 2C DNA content decreased gradually with increasing the concentrations, while those having 4C DNA increased.

Trigonella foenum extracts not affected on PMCs. Of *P. sativum* plant after the seeds treated with 0.5-1% and storage for three months but, significant percentage of chromosome abnormalities observed in the PMCs of the plants treated with 2% *Trigonella foenum* extracts and storage for 3 months. After storage for 6 months, all treatments have significant effect on abnormalities of PMCs. In all the treatment, various abnormal cells observed in mitotic and meiotic division. Aberrant cells observed according to concentration and time of storage of *Trigonella foenum* extracts.

These results clear that the fenugreek extracts with low concentration in storage the seeds are use safely.

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