RESEARCH ARTICLE

AN EVALUATION OF CYTOTOXIC POTENTIAL OF *TRIGONELLA FOENUM GRAECUM* USING *ALLIUM CEPA* ROOT MERISTEMS

Shalini Saxena*

Lab. of Cytogenetics, Department of Botany, Bareilly College, Bareilly, U.P. India 243001
Email: 2126Shalini@gmail.com

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Abstract: The cytotoxic potential of *Trigonella foenum graecum* (fenugreek) a common spice, was investigated using *Allium cepa* root meristem. Stock solution was prepared by using serial dilution method and 100ppm, 250ppm, 500ppm, 750ppm and 1000ppm conc. were prepared. Root meristems of *Allium cepa* were treated with above mentioned conc. of Fenugreek for 2, 4 and 6 hours. Result revealed mitodepressive behaviour of the spice and it also caused various chromosomal aberrations. Disturbed metaphase, chromatid separation, breakage at metaphase, scattered metaphase stickiness of chromosomes and rings were predominant aberrations induced by Fenugreek. Polarity abolition, Chromatin Bridge and laggards were reported at anaphase. Mitodepressiveness of *Trigonella* is due to the presence of a steroidal substance Diosgenin which is used as a starting material in the synthesis of sex hormones and oral contraceptives. Excessive and indiscriminate use of fenugreek may cause abortion in females hence its overdosing should be checked and scientific dose must be prescribed. At the same time Diosgenin extracted from *Trigonella* seeds inhibits spindle formation in dividing cells and for this reason it can be successfully used in the treatment of cancer.

Keywords: Cytotoxicity, Chromosomal aberrations, Root meristems

INTRODUCTION

Trigonella foenum-graecum commonly known as fenugreek is cultivated for its medicinal properties for many centuries. The seeds and leaves of this plant are used for the diabetes in many traditional systems including Ayurvedic medicine (Nahas and Moher, 2009).

Fenugreek seed contains 23.06% gums,28.00% mucilage, moisture 6.3%,protein 9.5%,fat 10.0%,crude fibre 18.5%, carbohydrate 42.30%,ash 13.40%,calcium 1.3%,phosphorus 0.48%,iron 0.011%,sodium 0.09%,potassium 1.7%,vitamin A 1040IU/100gm,vitamin B, O.41mg/100gm, vitamin C-12.0 and Niacin 6.0mg/1100gm. Calorific value 370calorie/100gm.

Fenugreek seeds are a very good source of protein; in a nutshell fenugreek seeds contain many substances like protein, starch, sugar, mucilage, mineral matter, volatile oil, fixed oil, vitamins and enzymes. Seeds are rich in essential amino acids. Raw dry seeds contain about 150 mg of trigonelline and practically no nicotinic acid.

If the seeds are roasted sufficiently to brown then about 2/3 of the trigonelline is converted in to niacin or nicotinic acid (vitamin A). Fenugreek has been used both as a food additive and as medicine. As a spice fenugreek seeds also add to the nutritive value and flavour of foods. Seeds are also used in colic, flatulence, dysentery, diarrhoea, dyspepsia with loss appetite, chronic cough, dropsy, enlargement of liver and spleen, rickets, gout, and diabetes. The seeds are used as carminative, tonic, aphrodisiac and an infusion given to small pox patients as a cooling drink, roasted and then infused, used in sweets served to ladies during the post natal period. Recent studies in England indicated that fenugreek seeds substantially contain the steroidal substance diosgenin, which is used as a starting material in the synthesis of the sex hormones and oral contraceptives. Seeds are also used by Indian women for its power to promote lactation.

A lot of work has been done to find out the antifungal, antibacterial antimicrobial and anti inflammatory properties of spices. Flamming *et. al.* (2004) studied genotoxicity testing of fenugreek extract. In this study genotoxicity of fenugreek extract containing greater than or equal to 40% 4 hydroxyisoleucine (an active ingredient for blood glucose control) was investigated as a part of a safety evaluation of novel food ingredients for use in the control of blood glucose. Similarly Mai mohammad *et.al* (2013) studied in vitro cytotoxic activity of seed oil of fenugreek against various cancer cell lines. The results show that fenugreek seed oil significantly reduced the all viability and altered the cellular morphology in a dose dependent manner.

These results were also in conformity with that of Abdulazir Alsemari *et.al* (2014). Various normal and cancer cell lines were exposed to fenugreek extract at differing concentration (100mg/ml, 200mg/ml and300mg/ml) and at different time points (0, 24, 48, 72 and 96hrs) .Aqueous fenugreek seed extract a meliorates adriamycin –induced cytotoxicity and testicular alteration in albino rats (Sabre Asaka *et.al.*2012). They observed that treating animals with ADR and aqueous seed extract of fenugreek led to an improvement in the cytogenetic effect and testicular alternations induced by ADR.

*Corresponding Author

Keeping all these facts in minds in the present paper an attempt had been made to evaluate the cytotoxic potential of *Trigonella foenum-graecum* using plant chromosomes as a test assay.

MATERIALS AND METHODS

To obtain stock solution 1gm fine powder of seeds of fenugreek was dissolved in 1000ml distilled water and required concentrations – 100ppm,250ppm,500 ppm,750 ppm and 1000 ppm were prepared by dilution method. On the basis of the suitability for cytological studies *Allium cepa*, had been selected for investigation. Root meristems were treated with above mentioned concentrations for 2,4and 6 hours. After the treatment, the root tips were cut carefully and fixed in Carnoy's fluid (1:3 acetic, alcohol) for 24 hours and then transferred to 70% alcohol for preservation. The root tips were hydrolysed in 1N HCl for 3 minutes and squashed in 2% Acetocarmine for cytological studies. The slides were temporarily sealed, examined and microphotographs of selected aberrations were taken.

Parameters choosen for the studies- 1-The cytotoxic potentiality of fenugreek had been studied using macroscopic parameters.

- Time of root initiation after treatment of aqueous solution of undertaken spices.
- No of roots after treatment
- Root length(after three days)

2- The cytotoxic potentiality of fenugreek had been studied using microscopic parameter-mitotic index and percentage and frequency of nuclear and chromosomal aberrations

Mitotic index= No. Of dividing cells*100/total no, of cells

3-Mitotic index, frequency of chromosomal aberrations and their percentage were calculated using the method of Mousa (1982).

4-To study chromosomal aberrations and nuclear aberrations iron-alum Acetocarmine technique (Godward, 1948) was used.

5- The significant aberrations in each were micro photographed using NIKON MICROPHOT-FXA MICROPHOTOGRAPHIC EQUIPMENT.

RESULTS

Results indicate that aqueous seed extract of *Trigonella foenum graecum* was mitodepressive. Decline in mitotic index was directly proportional to the duration and concentration of the treatment. As duration and concentration of the treatment was increased, mitodepressiveness increased. In controlled condition mitotic index of *Allium cepa* was 24.74%. A treatment of 100 ppm aqueous seed extract of *Trigonella foenum graecum* reduced mitotic index to 9.50%(2hrs),9.12%(4hrs) and 9.36%(6hrs). Similar trends were observed during 250ppm, 500ppm and 750ppm treatment. Abrupt fall in mitotic index was observed during 1000ppm treatment. The percent value were 6.71 %(2hrs), 6.06 %(4hrs) and 6.04 %(6hrs). Fenugreek also induced various chromosomal and nuclear aberrations. At prophase fenugreek induced aster formation and nuclear disintegration. Metaphase was supposed to be most susceptible to the treatment of aqueous seed extract of fenugreek, as six types of chromosomal aberrations were observed at metaphase. Disturbed metaphase, chromatid separation, breakage at metaphase, scattered chromosomes, stickiness of chromosomes and rings were observed. At anaphase polarity abolition, extrusion, Chromatin Bridge and laggards were reported. At telophase no aberrant cells were recorded (table1.1).

DISCUSSION

The role of environmental carcinogens in human cancer has been the subject of considerable scientific research. Dietary factors are now considered to be the most important risk determinants for human cancer (Gori 1979, Weisburger *et.al*.1977). Recently it has been observed that during cooking of food many heterocyclic compounds are formed. Among these compounds are some of the most potent bacterial mutagens known (Bjeldanes *et.al*.1979). Many of these mutagens have proven to be multipotent carcinogens in mice.

Toxicological evaluation of fenugreek seeds was made in 60 diabetic patients in a 24 week study (Sharma *et.al*.1996). Clinical signs body weight, serum parameter, alkaline phosphatise, bilirubin creatinine and blood urea were studied and found that the patients ingested an experimental diet containing fenugreek seed powder (25gm/day), no renal or hepatic toxicity was observed. But blood urea concentration decreased after 12 weeks of treatment. Decline in mitotic index was also reported by Ene-obong and Amadi (1987) in *Boerhaavia diffusa* and *Vernonia amygdalina*; Kaushik (1993) in *Datura stramonium*; Medeiros and Takahashi (1887) in *Luffa operculata*; Alam *et.al*(1987) in *Ipomoea carnea*; Mogili and Vidyavati(1985) in *Ephedra*; Mogli *et.al*.(1984) in *Periwinkle roseus* and Shehab and Adam (1983) in *Anastatica hierochuntica.* Reason for mitodepressive effect of various chemical compounds, medicinal plants etc were given by various investigators differently. Zakia *et.al*.(1990) suggested that the reduction in mitotic activity takes place due to the inhibition of DNA synthesis, which in turn induced a substantial mitotic delay. The mitodepressive behaviour of aqueous extract of fenugreek seeds may be due to the presence of DIOSGENIN (an active ingredient). Aqueous seed extract of fenugreek caused breakage of chromosomes and scattered chromosomes at metaphase. Similar results were reported by Bhalla *et.al.* (1973). They reported the scattering of

chromosomes at metaphase during the study of induction of mitotic abnormalities in onion root tip cells by tobacco smoke condensate. Lagging in *Allium sativum* root meristems were reported by the treatment of food dyes (Bhalla 1998). Clove, red chillies, black pepper, nutmeg (Yadav, 2001) and sodium salicylate (Briand and kapoor, 1989). Lagging chromosomes are the fragments or daughter chromosomes left over on equatorial plate after anaphase, which lack centromere and do not connect to any of the poles but remains in the cytoplasm and later form micronuclei.

Sigenaga (1949) attributed that the lagging chromosomes, scattered chromosomes and chromosomal bridge are the result of hydration and dehydration process of spindle and chromosomes. Sometimes lagging chromosomes interfere with the process of cytokinesis and lead to binucleate cells.

CONCLUSION

During the course of investigations *Trigonella foenum-graecum* had been tested on *Allium cepa* root meristems for 2, 4 and 6 hrs and compared with control values. Macroscopic and microscopic both parameters had been studied but greater emphasis had been given to microscopic parameters-mitotic index and chromosomal aberrations, Aqueous seed extract of fenugreek proved mitodepressive when tested on root meristems of *Allium cepa* and also induced various chromosomal aberrations.

Recently use of fenugreek seeds increased due to its hypoglycaemic nature but as the results indicate that its higher concentrations were strongly mitodepressive, its overdosing must be checked, and a scientific dose must be prescribed. A closer look is necessary because of its potential mutagenic and cytotoxic behaviour and present work will definitely open many new approaches for the research. Investigator does not claim herself to be perfect and complete in the discussion of the subject as fragmentary literature is available but she does claim that she is very close to the perfection and thus in vitro effect of fenugreek as a substance with significant cytotoxicity to cancer cells points to the potential usefulness of fenugreek in the prevention and treatment of cancer.

Macroscopic Results

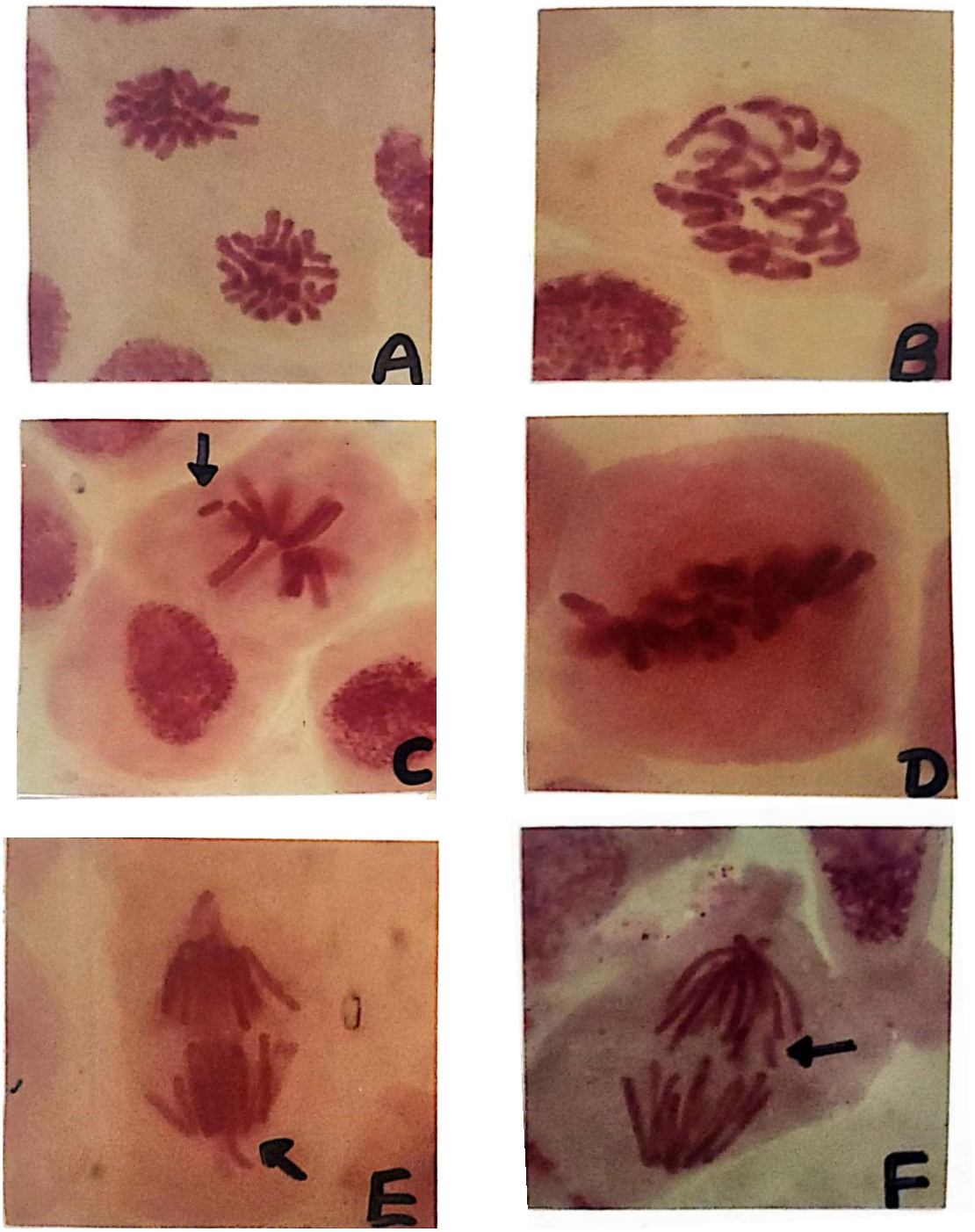
Table 1. Effect of aqueous seed extract of *Trigonella foenum graecum* on macroscopic parameters of *A. Cepa*.

S.N.	Parameters	Control value	Treatment of *Trigonella foenum graecum* in ppm				
			100	250	500	750	1000
1.	Root length (in cm)	3.2 cm	3.1	3.1	2.6	x	x
2.	No. of days of initiation of roots	5	6	6	7	x	x
3.	No. of roots per Onion bulb	7	7	5	5	x	x

Table 2. Mitotic index, frequency of aberrations and their percentage as induced by aqueous seed extract of *Trigonella foenum graecum* on *Allium Cepa* (2n = 16)

Concentration	100 ppm				250 ppm				500 ppm				750 ppm				1000 ppm				CONTROL
Duration (in hrs)	2	4	6	% of abr. (Conc. wise)	2	4	6	% of abr. (Conc. wise)	2	4	6	% of abr. (Conc. wise)	2	4	6	% of abr. (Conc. wise)	2	4	6	% of abr. (Conc. wise)	
Mitotic index (in %)	9.50	9.12	9.12		9.01	9.43	11.83		9.45	8.59	9.12		8.49	13.10	7.07		6.71	6.06	6.04		24.74
Types of aberrations	—	—	—		—	As	As	11.30	As	As	—	10.56	—	—	As	14.50	—	—	As	13.14	NO ABERRATION
	—	—	—		—	—	ND	10.24	—	ND	ND	9.07	—	—	ND	15.86	—	ND	ND	15.22	
	Dm	Dm	Dm	21.40	—	Dm	Dm	12.50	—	—	Dm	14.20	Dm	—	Dm	30.00	Dm	Dm	Dm	23.00	
	—	—	—		—	—	—		—	Chs	Chs	14.20	—	—	Chs	20.78	Chs	—	Chs	15.38	
	—	—	Br m	14.42	—	—	—		—	Br m	—	7.14	—	Dm	Br m	20.00	Br m	Br m	Br m	23.05	
	SC m	—	SC m	7.14	SC m	SC m	SC m	6.25	—	Sc m	—	21.40	—	—	Sc m	10.00	Sc m	—	Sc m	7.69	
	—	—	—		—	—	—		St m	St m	—	7.14	—	—	St m	10.56	—	—	—		
	—	—	—		—	—	—		—	—	—		—	—	—		Rm	—	—	12.76	
	—	PA	—	5.26	—	—	—		PA	—	—	11.11	—	—	PA	10.23	—	PA	PA	8.33	

	—	—	—		—	—	—		—	—	—		—	—	—		—	Ext A	Ext A	16.66	
	—	—	—		—	—	—		—	Lag A	Lag A	5.55	—	—	—		—	—	—		
	Bri A	—	—	11.76	—	Bri A	—	17.64	—	—	—		—	—	—		—	—	—		



Photoplate -1

Chromosomal and nuclear aberrations in *A. Cepa* as induced by aqueous seed extract of Trigonella foenum graecum.

A. Aster formation (Delay in cytokinesis)
B. Disturbed metaphase
C. Breakage at metaphase
D. Stickiness of chromosomes at metaphase
E. Extrusion at anaphase
F. Laggard at anaphase

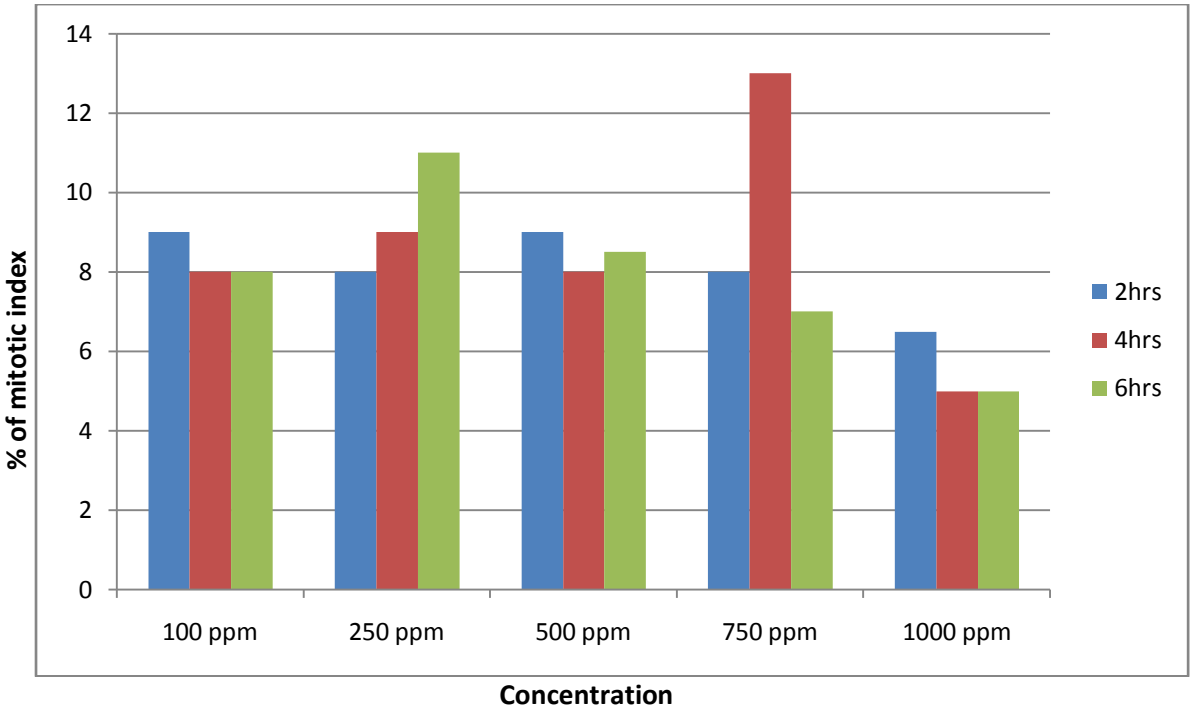


Fig. 1. Graphical representation of mitotic index of *Allium cepa* as induced by aqueous seed extract of *Trigonella foenum graecum.*

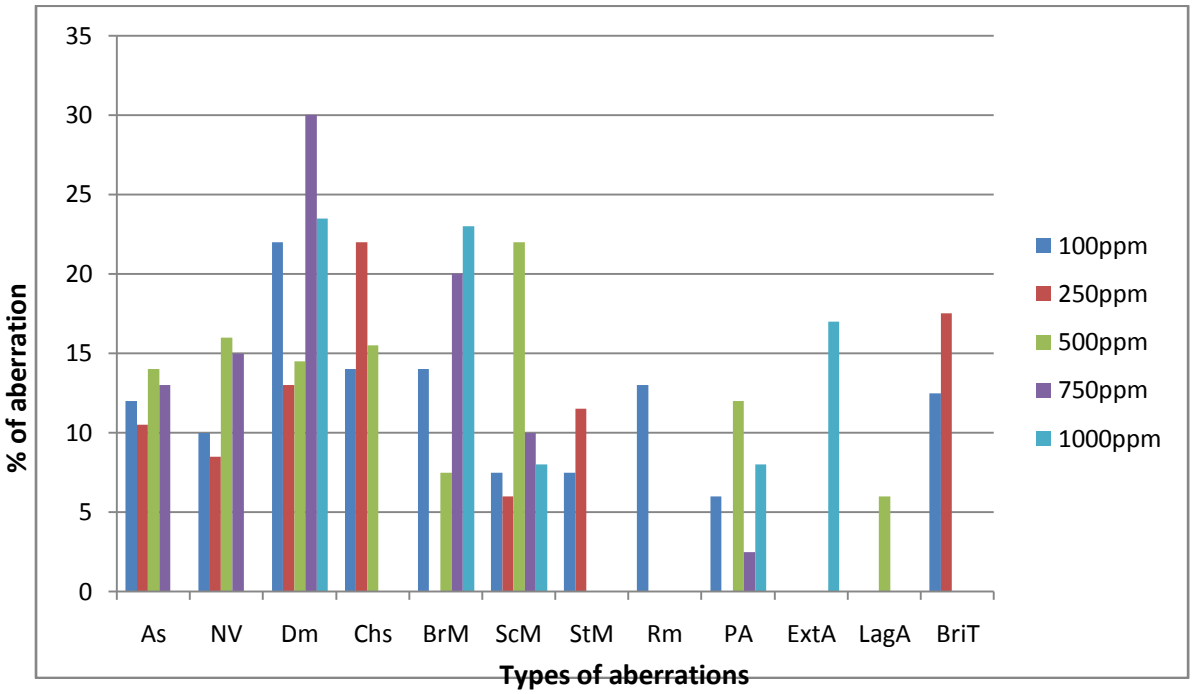


Fig. 2. Graphical representation of various nuclear and chromosomal aberration of *Allium cepa* as induced by aqueous seed extract of *Trigonella foenum graecum*.

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