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Effect of Some Common Herbicide on Root Tip Cells of *Trigonella foenum graceum* (Fenugreek)

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Abstract: ***Herbicides are chemicals used to manipulate or control undesirable vegetation. For study Maleic hydrazide were chosen as herbicide because it is applied to onions in the field at a rate of 2.2kg/ha when the bulbs are mature. It is widely known as plant growth regulator, used to suppress growth and induce dormancy in some crops and mostly used to reduce sprouting in stored crops. In the present investigation, the seeds of Trigonella foenum graceum were treated with four different concentrations (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) of maleic hydrazide to observe the cytological effect on them. The treatment was given at the seed level. Various abnormalities were noticed at cytological level in different concentrations. Fragmented nuclear material, Sticky metaphase, Sticky Anaphase, Vacuolated cells, Elongated cells, Binucleated, Disrupted Cells were the most frequent abnormalities. The decrease in germination rate was observed in 10^{-3}. Maximum abnormalities were observed in 10^{-3} and 10^{-4}.***

Keywords: Mitotic Index, Total No. of Aberrant cells, Standard Error (SE), Mean

1. Introduction

Fenugreek (*Trigonella foenum graecum L.)*, belongs to family Fabaceae, an annual cash crop of India, commonly known as "methi", is cosmopolitan in distribution and is a well known economic herb which produces small and yellowish brown seeds. The seeds are used as condiment; young plants serve as vegetable for human consumption. They are believed to be the native of Mediterranean region, and now widely cultivated in most parts of the world. It can also be efficiently incorporated into short-term crop rotations in western Canada. Fenugreek has been reported to generate a high yield of good quality forage and hay or silage.

In addition, the small inconspicuous flowers from fenugreek had a 4% success rate when crossed, making hybridization a less attractive route for crop improvement. Mutation breeding has become increasingly popular in recent times as an effective tool for crop improvement. Some legume crops that have been improved through mutation breeding are soybeans, string beans and French beans, navy pea beans and haricot beans, peas and fenugreek and lupines. A majority of induced mutations in these plants are recessive, and can be observed to segregate in a 3: 1 ratio in diploid crops like fenugreek.

2. Classification

Botanical Name: *Trigonella foenum graecum*
Chromosomes = 16

Kingdom	Plantae
Division	Magnoliopsida
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Subfamily	Faboideae
Genus	Trigonella
Species	foenum graecum

Maleic Hydrazide

Introduction

- Maleic hydrazide is a pyridazine that inhibits the synthesis of nucleic acid and proteins.
- It is a well known clastogenic agent in plants that induces chromosomal aberration and sister chromatid exchanges in root tip cell.
- Maleic hydrazide is a plant growth regulator used to suppress growth and reduce dormancy in some crop.
- It is mostly used to reduces sprouting in stored crops, such as potatoes, onion etc.
- It is regulated in crop to ensure correct use and to minimize consumer exposure.

Maleic Hydrazide	
Formula	4422
Density	1.6 at 770 FZ^{-1}
Molar Mass	112.09g/molH
Melting Point	+5720 C
Odor	Odorless
Boiling Point	477.2 +/ -25.0° C

Cytotoxic Effect of Maleic Hydrazide

1) Suppression of sprouting of vegetables and stored food crops.
2) Control sucker growth on tobacco plants.
3) Retardation off lowering, Prolongation of dormancy.
4) IV. It acts as an inhibitor of the synthesis of nucleic acid and protein.
5) Plants can break down Maleic Hydrazide into several products, one of which is Hydrazine, is a well known mutagen and carcinogen.
6) It does not seem to be toxic to bacteria and fungi.
7) The compound is regarded by soil microflora and hence can be utilized as a source of nitrogen nutrition.
8) It proved to be low toxicity to mammals, but in some instances it decreases the fertility of rats.

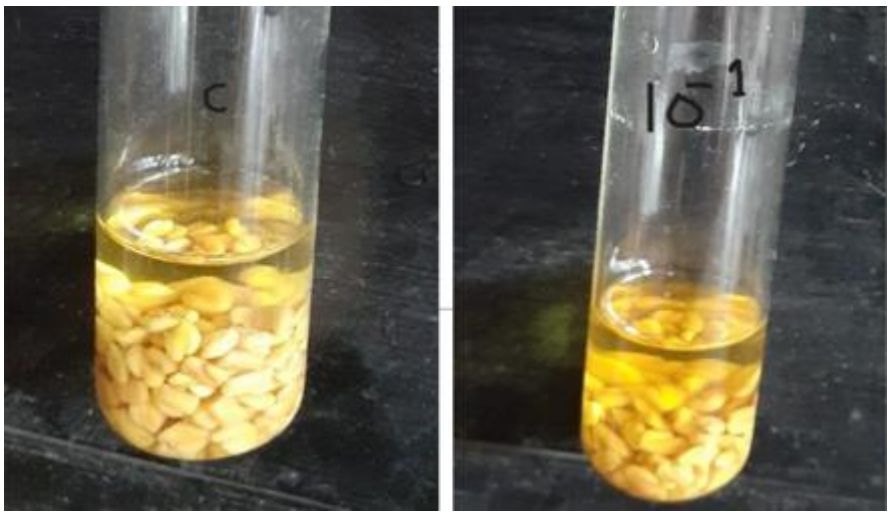
The present work was designed to study the cytotoxic effect of the MH herbicide using *Trigonella foenum graecum L.* as a biological system.

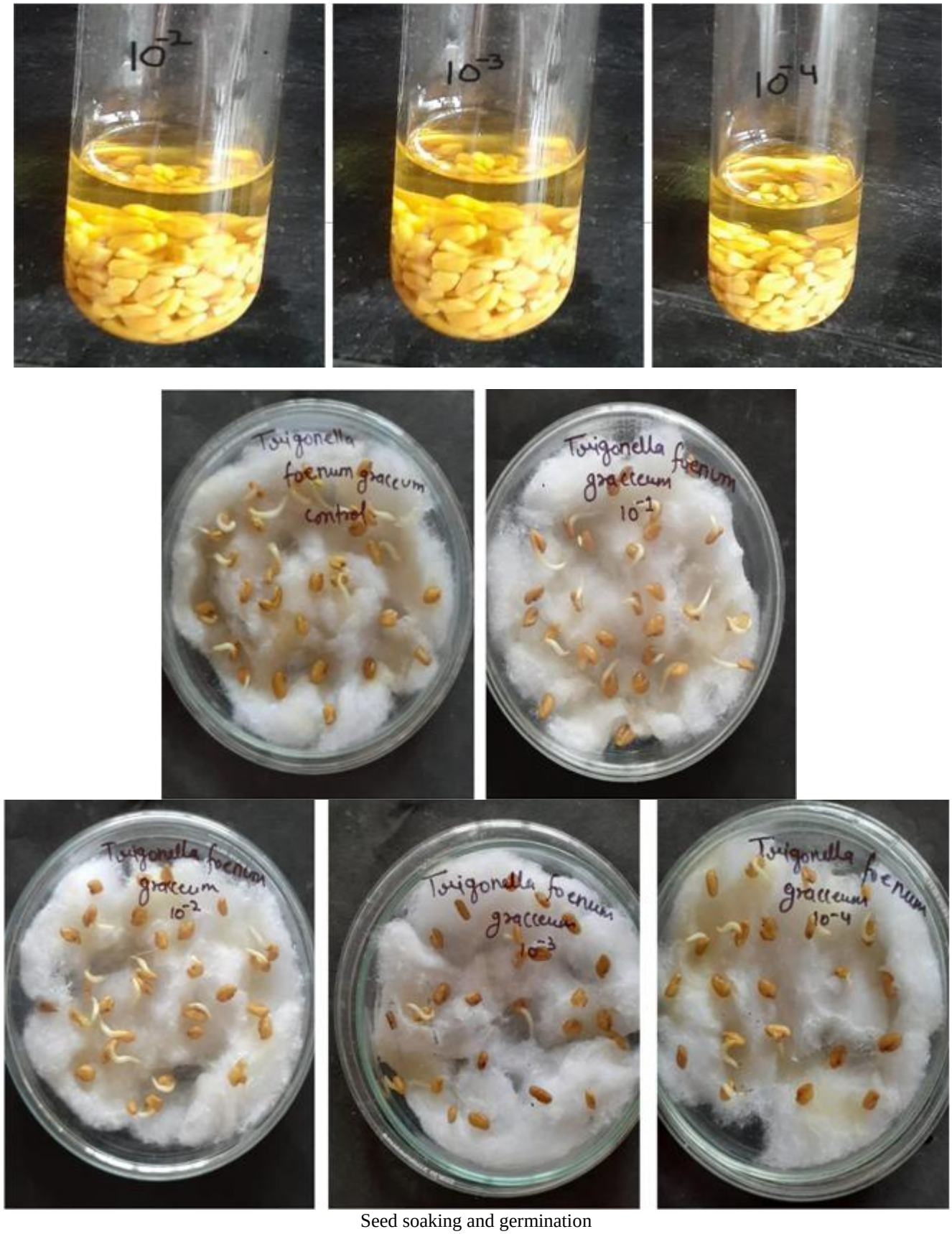
3. Materials

Equipment	Distilled Water	Materials
Microscope (connected with Laptop)	Di basic sodium phosphate	Brown paper
Weighing Machine	Mono basic sodium	Trigonella seeds
	phosphate Maleic	pH paper
	Hydrazide Acetocarmine	Conical flask
	Powder	Measuring Cylinder
	Para di chloro benzene	Petri dish
	Glacial Acetic acid	Test tube
	Ethanol	Sprit lamp
		Cover slip
		Slide
		Petri plates
		Filter paper
		Cotton

4. Methods

- Dry, healthy and properly washed seeds of *Trigonella foenum graecum* were presoaked in buffer solution for 2 hour, treated with Maleic Hydrazide, MH at 4 different concentration 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} with the help of serial dilution.
- It was prepared in sodium phosphate buffer with 7.0 pH for 8 hour with constant intermittent shaking.
- One set of seeds were kept untreated to act as control for comparison.
- Twenty Five seeds were used from each dose and control.
- In order to find out the effect of MH on root tip cells, Twenty Five seeds were spread Over moist cotton in Petri plate and allow to germinate in room temperature.
- After germination, when the size of root tip was reach into 2-3cm then root tips where excised.
- Excised root tip were pre-treated with 0.02% para-di-chloro benzene for 2h, and then washed with distilled water, fixed in glacial acetic acid: ethanol 3: 1 for 24 hours, again washed with distilled water and stored in 70% alcohol.
- For cytological observation the root tips were allow to heat for 25 to 30 min in 1.5% acetocarmine solution for taking stained.
- After that, temporary slides were prepared and slide was ready to observe.
- The Mitotic slides were observed under 40x and photograph were taken, separately for each concentration.





Seed soaking and germination

Cytological Observation

- Under microscopic observation, the cell of different concentration where observed, and they show different cellular abnormality.
- The mitotic index in control was observed 74.24 and gradually decreased from 68.96 to 45 % from 10^{-1} to 10^{-4}.
- In control, the chromosomes where observed normal, but the nature of chromosome where changes from low to

high concentration. Chromosomes and normal arrangement of cells were observed in control.

- Sticky Chromosomes where observed in all concentration.
- Sticky Metaphase, Sticky Anaphase were observed in 10^{-3}, Karyorrhexis were observed in all concentration except 10^{-2}, binucleated and un orientation of cells were observed in all concentration except 10^{-1}. Pulvraisedprophase were observed only in 10^{-2}.
- Vacuolated cytoplasm was observed in all concentration except 10^{-3}.

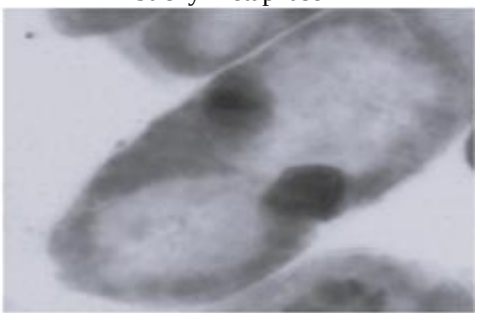
•Blebbing of cells, Ghost cells and elongation of cell shape were observed in all concentration, shape elongation and circularization of cells increase from lower to higher concentration.

5. Observed Photographs

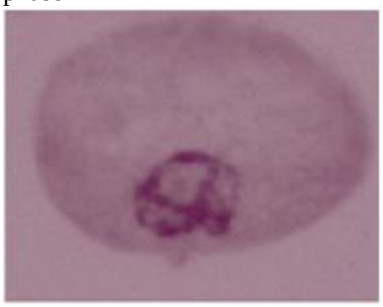
Chromosomes

Sticky Metaphase

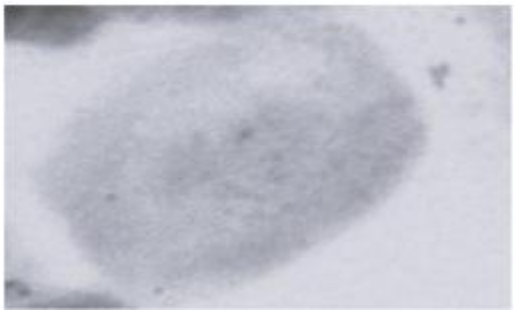
Sticky Anaphase



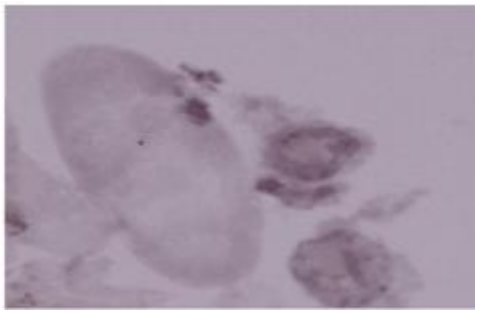
Binucleated



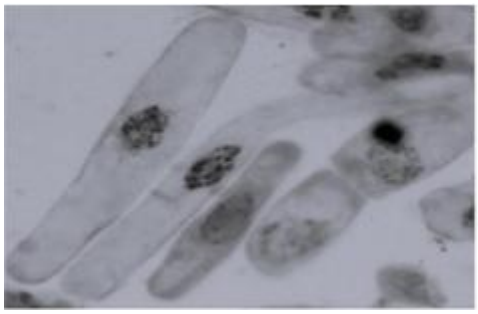
Cell Blebbing



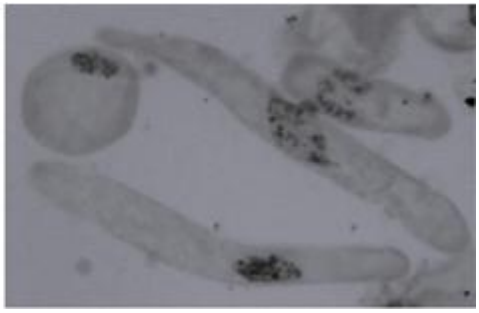
Ghost Cell



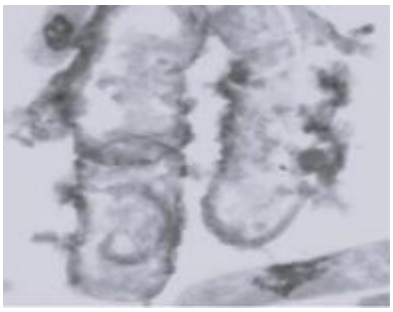
Nuclear Material going Outside from Cell



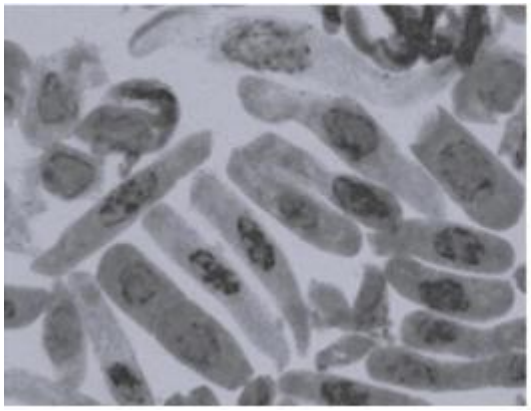
Elongated Cells



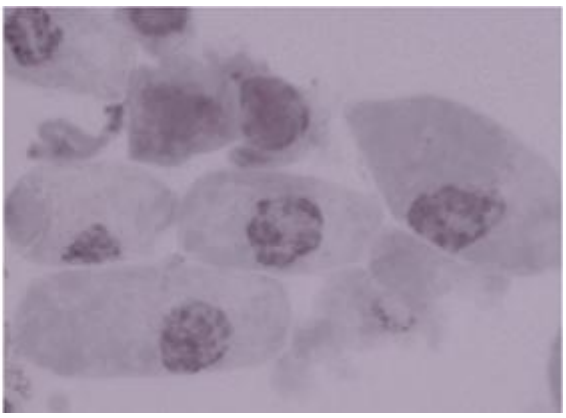
Circularization of cell



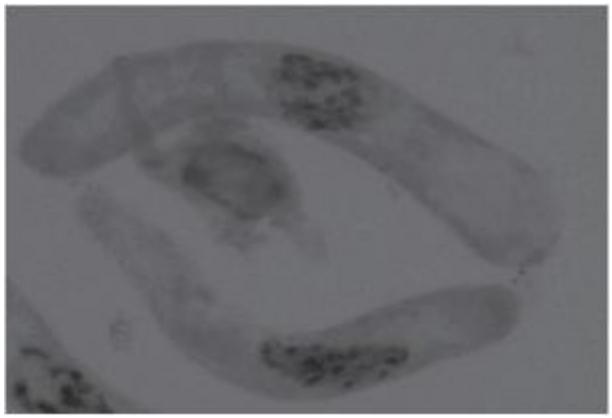
Cell Disruption



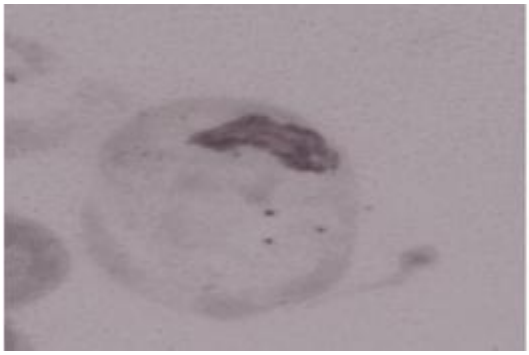
Abnormal arrangement of cells



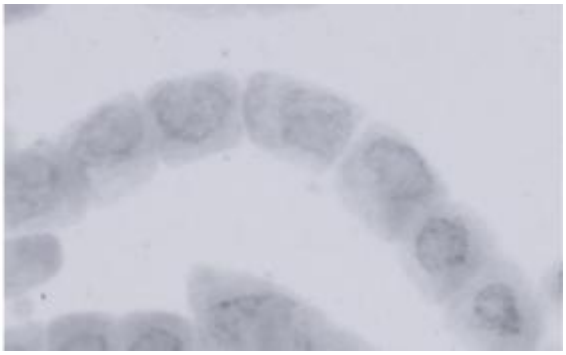
Pulvrised Prophase



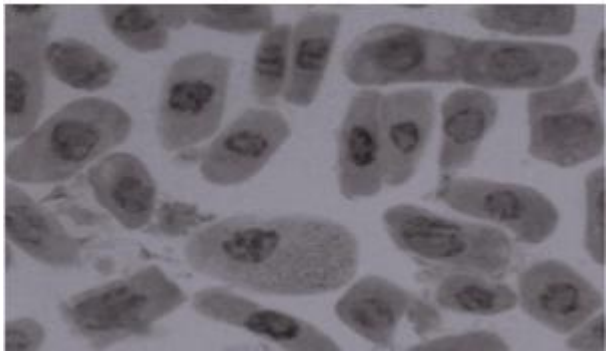
Abnormal shape of cell



Karyorrhexis



Normal arrangement of cells



Cell overlapping

6. Result

Effect of Maleic Hydrazide in chromosomes of root tip cells was observed under different concentration.

- The results were calculated with the help of Mitotic index.
- Mitotic index was calculated for 4 different concentrations for 12 hours along with control using the formula.

$$MI = \frac{\text{Total Number of Dividing cell}}{\text{Total Number of Cells Observed}} X\ 100$$

Actively dividing cells are the cells of metaphase and anaphase stage.

$$TAB = \frac{\text{Total Number Of Abberent Cells}}{\text{Total Number Of Actively Dividing cells}}$$

$$\text{Standard Error of mean (SE)} = \frac{\text{d}}{\text{N}}$$

Where SE = Standard Error
N = Sample Size
d = Standard Deviation

Table 1: Mitotic index and Frequency of chromosomal aberration at different stages of division in the root tip cells of Trigonella foenum

		No. of Dividing cells At different stage			
Concentration	No. Of cells observed	Metaphase	Anaphase	Telophase	MI% Mean ±SE
Control	66	15	18	16	74.24 ± 0.24
10^{-1}	58	10	19	11	68.96 ± 0.23
10^{-2}	54	12	10	9	57.40 ± 0.19
10^{-3}	48	8	7	12	56.25 ± 0.18
10^{-4}	40	7	6	5	45.0 ± 0.15

Table 2: No. and Nature of different types of chromosomal aberration in root tip of Trigonella foenum

Concentration	Actively dividing cell	SM	SA	PP	PY	VAC	BN	UN	TAB %± SE
Control									0.00%
10^{-1}	40		2		1	2			12%
10^{-2}	31	4		5		3	2	2	51%
10^{-3}	27	2	1		4		3	5	55%
10^{-4}	18	1			1	4	2	3	61

Legends of Table 2 : SM = Sticky metaphase, SA = Sticky Anaphase, PP = Pulvrised Prophase, PY = Pyknosis, VAC = Vacuolated cytoplasm, BN = Binucleated, UN = Unoriented Cells

- The mitotic index in control was observed 74.24 and gradually decreased from 68.96 to 45 % from 10^{-1} to 10^{-4}.

7. Conclusion

This study shows the cytotoxic effect of Maleic hydrazide on the root tip cells of *Trigonella foenum graceum*. By the results observed in study, Maleic hydrazide were proved to be an efficient genotoxic and cytotoxic substance which causes significant changes to the chromosomes, as the treatment increases.

The treated cells were shows various abnormalities due to cytotoxic effect of maleic hydrazide. The abnormalities increase with the increase of treatment concentration, sticky chromosomes, vacuolated cytoplasm, enucleated cells, karyorrhexis etc.

Effect of some common herbicide on root tip cells of *Trigonella foenum graceum* confirms that the maleic hydrazide which is used as growth regulator and herbicide can cause very much abnormality to the chromosomes; it could also cause effect on animal chromosomes.

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Literature Review

FarhaJabee, and Mohammad Yunus K. ANSARI, Danish Sahab. Cytogenetics and mutation breeding Lab, Department of Botany, Aligarh Muslim University, Aligarh – 202002 U. P. INDIA. Studies on the effect of maleic hydrazide on root tip cells and pollen fertility in Trigonella foenum graceum L.

They found that the higher concentration of maleic hydrazide were highly mitotic, lower concentration produced various types of chromosomal damage like fragments, stickiness of chromosomes, clumped metaphase, vacuolated cytoplasm are also seen.

William F Grant. Chromosomes aberration in plant as a monitoring system. Environmental health perspectives volume 27, pp.37-43, 1978.

Grant reported the potential of higher plants as a first tier assay system for detecting chemical mutagens is evaluated; the use of plant tissue for studying the induction of chromosomal aberrations is one of the oldest, simplest, most reliable and inexpensive method available.

Winifred Compton, Bulletin of the Torrey Botanical Club, Vol.79, No.3 (may-June, 1952), pp.205-211. The effect of maleic hydrazide on growth and cell division in pisumsativum.

Schoene Maleic and Hoffman 1949, conducting preliminary experiment with hydrazide, found that the chemical has pronounced, but temporary inhibiting effect on plant growth effects on both were in order of the concentrations of the MH solutions but the effects on cell division did not occur simultaneously with the effect on total growth.

Moore, r. h. science Washington 1950 vol.112 pp.52-3 ref. bible.2. several effect of maleic hydrazide on plants.

Moore reported that with the increase in the concentration of MH, stunting of many species due to temporary suspension of stem elongation from terminal buds or the death of terminal buds and adjacent tissues, expansion of lateral buds, localized accumulation of anthocynins or other non-green pigments

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