



GENOTOXIC EFFECTS OF *OCIMUM BASILICUM* L AND *LEUCAS ZEYLANICA* L IN ROOT TIP CELLS OF *ALLIUM CEPA* L

VISHNU SHANKAR SINHA AND NANDJEE KUMAR*

Department of Botany, Tata College, Chaibasa, Jharkhand – 833202.

*Department of Botany, Magadh University, BodhGaya-823234.

The present paper mainly deals with the effect of aqueous leaf extract of two plant species *Ocimum basilicum* L and *Leucas zeylanica* L on the mitotic index and chromosomal abnormalities in the root tip cells of *Allium cepa* L. About 50 g of fresh leaves were grinded separately to get the mother solution of the leaf extract which was further diluted to different concentrations by adding required quantity of double distilled water. The root tips of *Allium cepa* L were treated with different concentrations viz 10%, 25%, 50%, 75% and 100% of the extract of *Ocimum basilicum* L and *Leucas zeylanica* L separately and also with double distilled water for the control for four hours at room temperature. The treated and controlled root tips were fixed in freshly prepared 1:3 acetobutanol and squashed in freshly prepared 2% acetocarmine solution. The frequency of cell division (mitotic index) and chromosomal abnormalities were noted in different mitotic stages of mitotic cell division of each treatment. The result showed that both *Ocimum basilicum* and *Leucas zeylanica* Leaf extracts are mito-depressive and inducing chromosomal abnormalities such as stickiness, fragments, bridges, laggards, micronuclei, disturbed metaphase /anaphase and micronuclei. The chromosomal abnormalities showed a linear correlation with the doses of the leaf extracts. It was also noted that the leaf extract of *Ocimum basilicum* L is more mitodepressive than *Leucas zeylanica* L, hence *Ocimum basilicum* may be considered as a potential antitumor promoting agent .

Key words: Genotoxic, *Ocimum*, *Leucas*, leaf extract, MI, Chromosomal abnormalities.

In recent years allelopathy has acquired a key role in studying the mutagenic / genotoxic / chromotoxic / cytotoxic effects on the living organisms. Various workers reported remarkable allelopathic effect of Neem (Sinha and Kumar 2005, Adegbite *et al.* 2009), Mustard (Kumar and Kohli 1987), *Lantana* (Mohanka *et al.* 2005, Deena and Thoppil 2006) *Parthenium* (Sinha 2009), Red pepper (John and Abraham,1991), *Ocimum* (Tijo and Thoppil 1998). Grant (1978) pointed out that plant chromosomes are sensitive indicators of environmental pollution and suggested that higher plant system appears to be an excellent indicator of the cytotoxic / genotoxic/ mutagenic effects of environmental mutagens; therefore the plant system must be accepted as a first tier assay for detection of the possible genetic damage resulting from the use of environmental chemicals.

Allium cepa L is an efficient test material for chemical screening and *in situ* monitoring for genotoxicity of environmental contaminants and has been widely used to study genotoxicity of many pesticides revealing that these compounds can induce chromosomal aberrations in root meristem of *Allium cepa* L (Thais *et al.* 2007). Patra and Sharma (2002); Leme *et al.* (2009) reported that *Allium cepa* has relatively large monocentric chromosomes with reduced number and are accepted as suitable test organism for the study of environmental mutagenesis.

MATERIAL AND METHODS

In the present investigation allelopathic impact of leaf extracts of *Ocimum basilicum* L and *Leucas zeylanica* L have been studied on mitotic index and chromosomal abnormalities in the root tip cells of *Allium cepa* L. *Ocimum basilicum* L is a common indigenous weed and is widely used in folk medicines as a tonic, vermifuge and mental fatigue. The main phytochemical ingredients are ascorbic acid, eugenol, geraniol, methyl chevicol (Sagar and Zaffar, 2003). *Leucas zeylanica* L is a common weed growing in the cultivation field of *Oryza sativa*, *Saccharum officinale* and is widely used in snake bites and jaundice. The main phytochemical ingredients are alkaloids, tannins, flavonoids, steroids, glycosides and saponins (Paul and Saha 2012).

Preparation of leaf extract solutions : To get the mother solution of leaf extract of each species about 50 g fresh leaves were grinded separately followed by filtration and were further diluted to different concentrations (viz. 25%, 50%, 75%) by adding required amount of distilled water. Doubled distilled water was taken as control.

Squash preparation : Actively growing root tips were treated with different concentrations of the leaf extract of the two species separately along with the

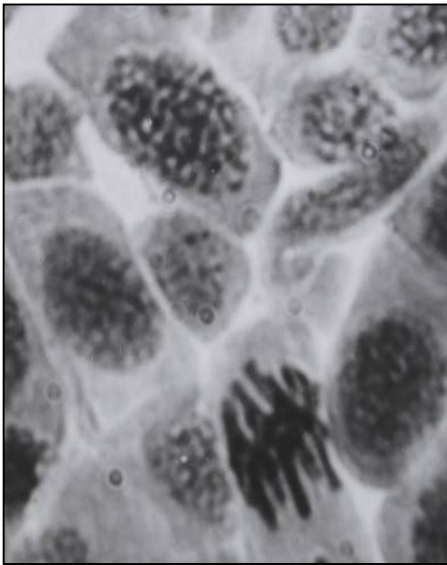


Fig:1.Stickiness

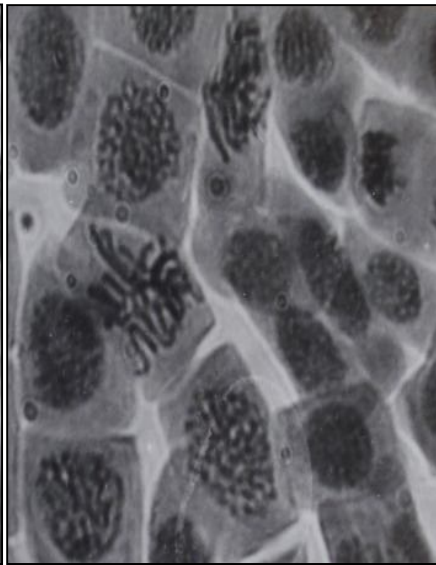


Fig:2.Fragments

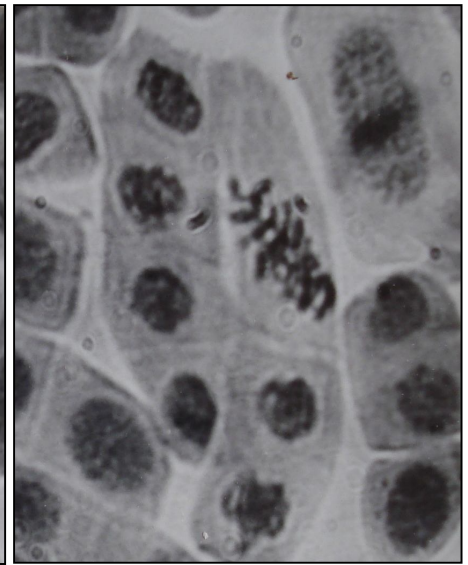


Fig:3.Disturbed

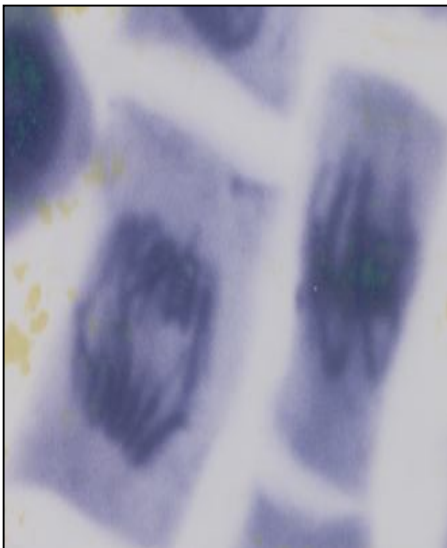


Figure: 4.Bridge

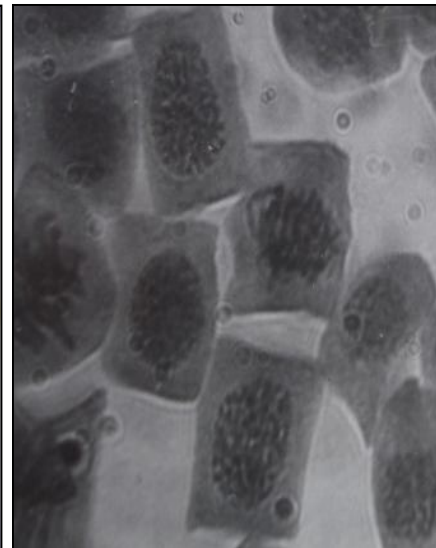


Figure:5.Micronuclei

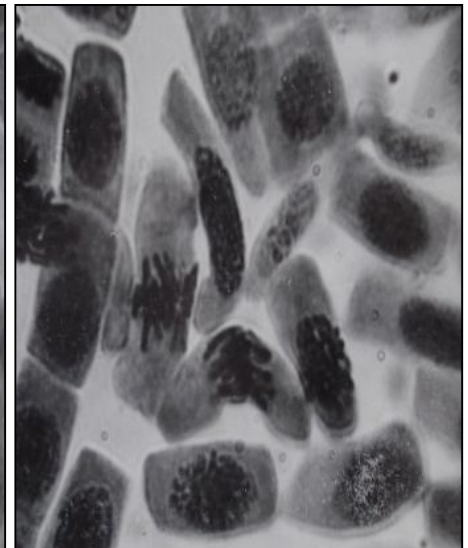


Figure:6.Disturbed Metaphase

control for four hours at room temperature. The root tips were fixed in freshly prepared 1:3 acetobutanol for 24 hrs and squashed in 2% aceto carmine to study the frequency of cell division and chromosomal abnormalities in each treatment. The mitotic index (MI) was calculated by number of dividing cells / total number of cells scored x 100 while percentage of chromosomal aberrations by number of abnormal cells / total number of dividing cells x 100.

OBSERVATION

In *Allium cepa* L the somatic number of chromo-

somes was noted to be 16. The cytological observations revealed that leaf extract of *Ocimum basilicum* L and *Leucas zeylanica* L showed depression of mitotic index but vigorously induced the chromosomal abnormalities such as fragmentations, stickiness, lag-gards, bridges, disturbed metaphase / anaphase, and micronuclei, however the chromosomal abnormalities were almost negligible in control (Fig 1-6). The depression of mitotic index was gradually decreased with increasing concentrations ranging from 15.11 ± 0.32 to 3.10 ± 3.10 in comparison to control (17.30 ± 0.32). The chromosomal abnormalities showed a linear correlation with the doses of the ex-

Table: 1. Effect of *Ocimum basilicum* L and *Leucas zeylanica* L extract on mitotic chromosomes on *Allium cepa* L.

Treatment (%)	Plants	MI %	% of chromosomal abnormal cells	Stickiness %	Fragment %	Disturbed metaphase/anaphase / Telophase %	Bridge %	Laggard %	Micronuclei and Binucleate %
Control	-	17.80±0.32	-	-	-	-	-	-	-
10%	L	15.11±0.32	6.50	2.50	1.20	1.05	0.87	0.63	0.25
	O	14.02±0.26	7.10	2.60	1.80	1.04	0.98	0.47	0.21
25%	L	12.21±0.65	12.10	3.36	3.02	1.90	1.60	1.58	0.64
	O	11.76±0.22	15.60	4.30	3.70	2.80	2.20	1.74	0.86
50%	L	08.62±0.36	18.38	4.89	3.82	3.50	2.80	2.20	1.17
	O	06.21±0.28	22.67	5.80	4.67	4.30	3.80	2.70	1.33
75%	L	06.50±0.16	26.40	6.30	5.40	5.30	4.30	3.50	1.60
	O	04.80±0.12	30.30	7.30	6.50	5.80	5.20	4.10	1.40
100%	L	04.68±0.62	34.21	8.76	7.60	6.20	5.24	4.10	2.30
	O	03.10±0.16	42.59	9.96	8.20	7.86	6.84	5.80	3.69

tracts ranging from 6.50 % to 42.59 %. Interestingly, the extract of *Ocimum basilicum* was more effective than *Leucas zeylanica* to induce the chromosomal abnormalities (Table-1).

DISCUSSION

Our results confirm the earlier reports on mitotic inhibition due to leaf extracts of different plants such as Neem (Sinha and Kumar 2005), *Ipomoea carnea* (Alam *et al.* 1987) *Chrysanthemum* (Sinha, 2003), *Lantana* (Mohanka *et al.* 2005), Black pepper (Abraham and John 1989), *Parthenium hysterophorus* (Sinha 2009). The mitotic inhibition might have been achieved due to blocking of DNA synthesis at S phase or arrest of G₁/ a prolonged G₂ phase of cell cycle (Sudhakar *et al.* 2001, Mohandas and Grant 1972, Tijo and Thoppil 1998, Soni *et al.* 1982), however Cumini *et al.*(1996) reported that the proteins which determine the duration of transition from metaphase to anaphase are concerned with the transformation of chemical energy into mechanical work of mitosis. Evidently the doses of leaf extracts of the two species and mitotic index (MI) showed an inverse correlation which is compatible with the hypothesis that inhibition of mitosis may be due to phytochemical ingredients.

Chromosomal abnormalities are considered as reli-

able indicators of mutational changes and are used as reliable evidence for screening the mutational activity (Kihlman 1963). The present result on the induction of chromosomal abnormalities due to leaf extracts confirm the earlier reports (Kaur and Grover 1985, Mohanka *et al.* 2005). Stickiness comprising a dominant abnormality may be considered a type of physiological adhesion involving mainly the protein matrix of chromatin material (Patil and Bhat, 1992), however Klasterska *et al.* (1996) suggested that stickiness arose due to improper folding of chromosome fibers. The fragments may be formed due to DNA breakage by endonucleases (Grant, 1978). Multipolar anaphase abnormalities are caused due to inhibition of spindle formation (Amer and Ali 1983). Bridges are the result of chromosomal breakage where as laggards due to stickiness of chromosomal ends (Kaur and Grover, 1985). Micronuclei might be due to the aggregation of chromatin materials into masses of various number and size. Omanakumari *et al.* (2006) reported that the micronuclei appeared due to fragmentations of chromosomes by the carcinogenic action of monosodium glutamate.

The higher potential of the leaf extract of *Ocimum basilicum* for mitotic inhibition and induction of chromosomal abnormalities might be due to the biochemical ingredients; however details may be achieved by further studies of impact of individual phytochemical.

REFERENCES

- Adegbite EA, Ayodele MS, Odunbaku KR and Idehen ED (2009) Mutagenic effect of neem leaf extract used in traditional medicine on *Allium cepa* L. *Scientific Research and Essay*. **1** 1315-1321.
- Amar S M and Ali E M 1983 Cytological effects of pesticides XIV. Effect of the insecticide dipterex "trichorphon" on *Vicia faba* Plant *Cytologia*. **48** 761-770.
- Cumminis J E Bllomquist JG and Rusch H P 1966 Anaphase delay after inhibition of protein synthesis between late prophase and pro metaphase, *Science*. **154** 1343-1344.
- Grant 1978 Chromosome aberrations in plants as monitoring system. *Env Health Pers*. **27** 37-43.
- John A T and Abraham 1991 Cytological changes produced by red pepper in mitotic cells of *Vicia faba* L *Caryologia*. **44** 325-331.
- Kumar A and Kohli R K 1987 Autotoxicity of Reg weed *Parthenium*. *Weed Sc* **15** 629-632.
- Kaur P and Grover I S 1985 Cytological effects of some organophosphorus pesticides *Cytologia*. **50** 187-197
- Kihlman BA 1966 *Action of chemicals on dividing cells*, Prentice -Hall Inc. Englewood, Cliffs, New Jersey.
- Klasterska I, Natarajan A T and Ramel C 1976 An interrelation of the origin of subchromatid aberrations and chromosome stickiness as a category of chromatid aberrations. *Hereditas*. **83** 153-162.
- Leme DM, Marin-Morales, MA 2009 *Allium cepa* test in environmental monitoring : a review on its application. *Mutation Research*. **682** 71-81.
- Mohandas and Grant 1972 Cytogenetic effect of 2,4-D amitole in relation to nuclear volume and DNA content in some higher plants. *Canad J Genet Cytol*. **14** 773-783.
- Mohanka R, Sinha V S and Kumar N 2005 Mutagenic potential of the extract of *Lantana camara* L XXVIII Conference of I B S, Oct 24-26, B S I, Dehradun , India.
- Omanakumari N, Shaiju P N and Rejitha P G 2006 Cytotoxic effects of the food additive *Ajinomoto* on root tip cells of *Allium cepa* L. *J Cytol Genet*. **7(NS)** 63-68.
- Patil BC and Bhat G I 1992 A comparative study of MH and EMS in the induction of chromosomal aberration on lateral root meristem in *Clitoria ternate* L *Cytology*. **57** 259-264
- Patra M and Sharma A 2002 Relative efficacy of *Allium cepa* and *Allium sativum* in anaphase – telophase test screening metal genotoxicity. *Biologia* **57** 409-414.
- Paul Swati and Saha Dibyajyoti 2012 Pharmacognostic studies on methanolic extract of the aerial part of *Leucas zeylanica* L. *Asian J Pharm Res*. **2(4)** 142-143.
- Sinha V S 2003 Comparative studies of the hazardous effects of the chemical and biological pesticides on *Vicia faba* L Ph D Thesis of Patna University.
- Soni N K Oswal P and Yadav S K 1982 Effect of solar eclipse on cell division in the meristematic root cells of *Allium sativum*. *Cytologia* **47** 353-358.
- Sudhakar R, Gowda KNN and Venu G 2001 Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa* *Cytologia* **66** 235-239.
- Sagar B P S and Zafar R 2003 Regulation of phenolic oxidation by ascorbic acid in callus cultures of *Eclipta alba* Hassk, *Indian drugs* **40** 570-572.
- Sinha VS 2009 *Parthenium hysterophorus* induced genotoxic hazards in *Allium cepa* L *J Nature Environment and Pollution Technology* **8** 725-728
- Sinha V S and Kumar N 2005 Neem an alternative to

synthetic pesticide XXX Annual conference of Environmental Mutagen Society of India (March 17-19,2005) pp- 85.

Tomkins DJ and Grant WF 1972 Comparative cytological effects of the pesticides Menazon, Metrobromuron and Tetrachloriosphthalonitrile in *Hordeum* and *Tradescantia*. *C and J Genet Cytol* **14** 245-256.

Tijo A and Thoppil J E 1998 Cytotoxic properties of *Ocimum americanum L* *Bull Pure Appl Sci* **17B** 49-53.

Thais C, Dania Elisa C, Maria A (2007) Pesticides Biochemistry and Physiology, Mechanism of micronuclei formation in polyploidized cells of *Allium cepa* exposed to trifluralin herbicide *Indian Botanical Society* **88**(3) 252-259.