INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES
IJPAES
Volume-4, Issue-2, April-June-2014
ISSN 2231-4490

Coden : IJPAES www.ijpaes.com
Received: 4th Feb-2014 *Revised: 28th Feb-2014* *Accepted: 28th Feb-2014*
Research article

CYTOGENETIC EFFECTS OF TILT ON ROOT TIP MERISTEM OF ONION *ALLIUM CEPA* L.

P.V.Pulate[a*] and J.L.Tarar[b]

[a]P.G.Department of Botany, Vidya Bharati Mahavidyalaya Amravati-444602, Maharastra, India
[b]Department of Botany, Institute of Science, Nagpur, Maharastra, India
*Corresponding author, e-mail: pvpbot2005@rediffmail.com

ABSTRACT: Cytotoxic effect of the fungicide tilt was investigated in root tip cells of *Allium cepa*. The seeds of *A.cepa* were treated with different concentrations (0.02%, 0.04%, 0.06%, 0.08 %) of Tilt for 3, 6, 9, 12 h treatment periods. The results showed that the mitotic indices gradually decreased with increase in time and concentration gradient. The abnormalities observed include C-metaphase, sticky metaphase, diagonal metaphase, disturbed metaphase, multipolar anaphase, precocious anaphasic chromosome, chromosome bridge at anaphase, laggard.

Key words: *Allium cepa*, mitotic index, chromosomal aberrations, genotoxic effect, fungicides.

INTRODUCTION

The uncontrolled presence of chemicals in ecosystem, the diversification of synthetic drugs and the use of pesticide in agriculture have become inescapable necessity. Fungicides are most commonly used against diseases of agricultural crops in many countries of the world. Although fungicide application results in quick and high control of the diseases, the widespread use of these chemicals may cause environmental and food contamination [1]. Environmental pollutants may be mutagenic or toxic for all living organisms [2,3,].Constant use of these chemicals may result in changing the hereditary constitution of the organism[4]. A large number of compounds have been identified for clastogenic, mutagenic and carcinogenic abnormalities usually induce genetic damage as well [5]. Studies indicate that indiscriminate use of agro-chemicals results in many undesirable secondary consequences in higher plants. A number of workers have carried out studies on cytological effects of different agrochemical on different plant species [6]. Generally toxic effects of environmental pollutants cause genetic damage on plant cells. But toxicity is not always correlated with genotoxicity [7]. The fungicide tilt (Propiconazole) is a systemic triazole fungicide with broad range specificity against fungal pathogens. It is a commercial form of triazole and is used extensively in the agricultural area. However, no study is available on the cytogenetic effects of this chemical in the plant systems. The aim of this study was to investigate the chromosomal abnormalities induced by fungicide tilt on the root tips of *Allium cepa* L. and also to determine the relation between mitotic index and chromosome abnormalities.

MATERIALS AND METHODS

Seed of *Allium cepa* L var. N-53 were obtained from National Horticultural Research and Foundation (NHRDF) chittagong, Nasik. Seeds (1,500) were presoaked for 12 h and treated in 50 ml of the test solution (0.02, 0.04, 0.06, 0.08) of tilt fungicide for 3, 6, 9, 12 h at room temperature (22+2C). Conical flasks containing the seeds and solution were periodically shaken for 2-3 min. during the treatment. After the treatment, seeds were placed on moist germinating paper in 5 petri dishes (100 seeds in each) and the embryonic roots were excised. Fixed in acid alcohol (acetic acid and alcohol, 1:3 v/v) and preserved in alcohol. Ten slides were prepared for cytological studies. Each concentration of test solution was screened by squash technique method and examined under microscope. Various types of abnormalities were recorded. The resulting data were pooled to calculate percentage of abnormalities and mitotic index (MI). Microphotographs were taken from selected semipermant slide. Abnormalities were photographed using a Zeiss Primo Star microscope mounted with Canon camera model, Power Shot A640.

RESULT AND DISCUSSION

The mitotic index is the reliable predictor of cell proliferation in the tissue. The mitotic index of control set with little reduction in mitotic index as the time of treatment prolonged. The treatment of Tilt fungicide on *Allium cepa* caused a dose-dependent decline in mitotic index. Careful screening in mitotic index was noticed in the root tip cells as concentration and duration of treatment increased. Mitotic index of control set was 11.4 ±0.37 in 3h, 11.88 ± 0.21 in 6h, 10.6 ± 0.72 in 9h, 9.3 ± 0.61 in 12h.From it declined to 11.10 ± 0.41 3h (0.02%) to 2.8 ± 0.46 12h (0.08%). The mitotic index values were slightly lower than that of the control values at lower concentration (0.02% and 0.04%) at 3h treatments. The MI of the other concentration were sharply decreased as the time of treatment increase recording value of 2.8±0.64 12h (0.08%) treatment (Table-1). The other types of chromosomal abnormalities have been given in the cytological plate from figures (1a-h).The increase in the concentration and time significantly increase abnormalities (% abn.) of each stage (Table-2) The detailed analyses of mitotic abnormalities recorded various types of aberrations. The highest value was recorded at the higher concentration and longer time of exposure (42.5% for 0.08% concentration in 12h treatment). The higher percentage of abnormalities was C-metaphase (Fig-1a) and Sticky metaphase (Fig-1b).The other type of the chromosomal abnormalities were diagonal metaphase (Fig-1c), disturbed metaphase(Fig-1d), multipolar anaphase(Fig-1e), precocious anaphasic chromosome(Fig-1f),chromosome bridge at anaphase(Fig-1g), Laggard(Fig-1h).

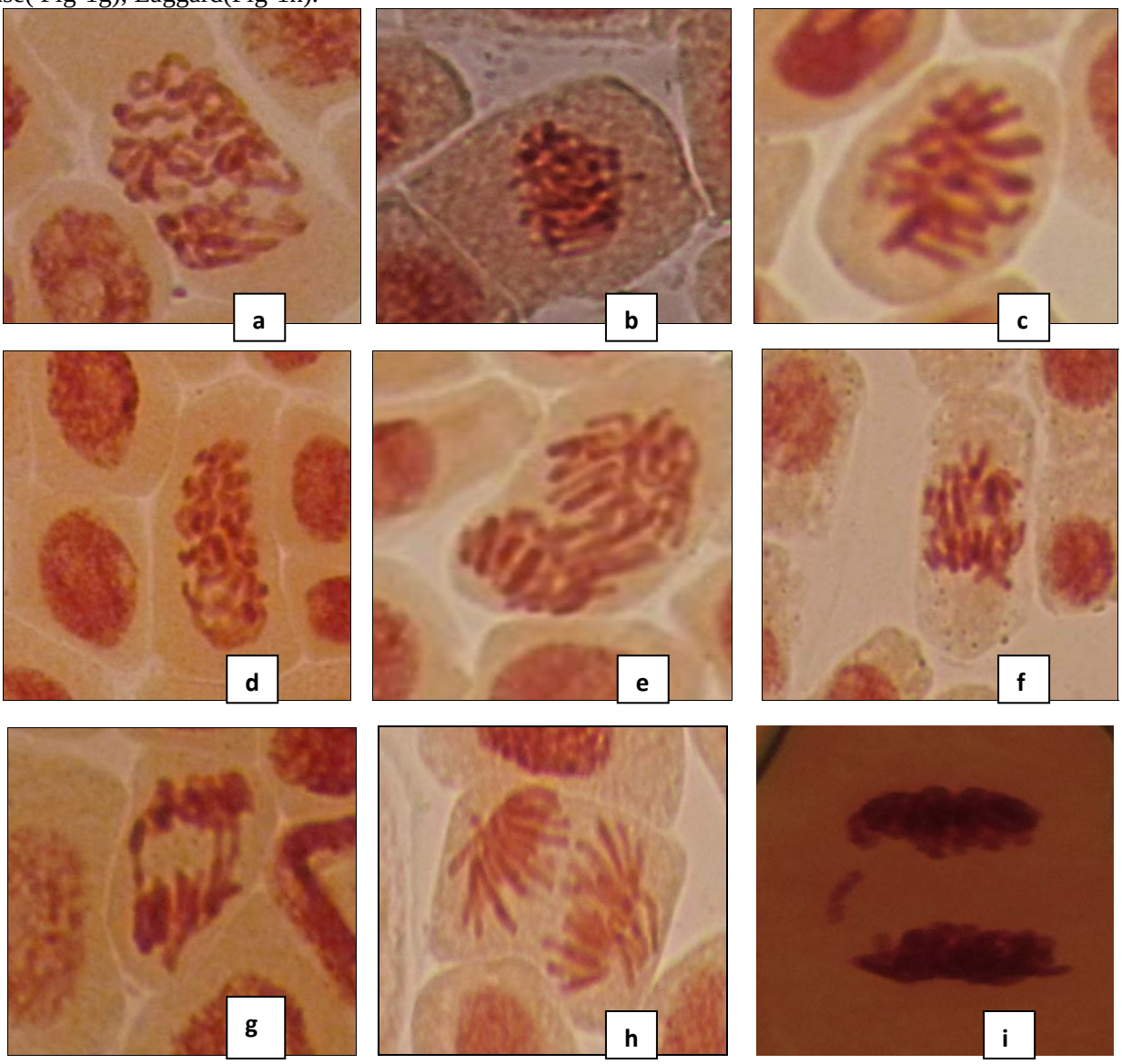

Fig.1-: Chromosomal Aberration cell induced by Tilt fungicide treatment in root tip cells of *Allium cepa*. a) C-metaphase, b)Sticky metaphase, c)Diagonal metaphase, d) Disturbed metaphase , e) multipolar anaphase, f) Precocious anphasic chromosome ,g) Chromosome bridge at anaphase, h)Precocious chromosome at telophase, i) Laggard.

Table 1.Effect of different concentration of Tilt fungicide on the mitotic index (MI), stage index and abnormalities (% abn) of each stage in root tips of *Allium cepa* L.

Treatment		MI ±S.E	Prophase		Metaphase		Anaphase		Telophase	
Time	Concentration		Index	%abn.	Index	%abn.	Index	%abn.	Index	%abn.
3h	Control	11.4 ± 0.37	43.1	0.9	28.2	1.2	16.4	0.7	12.3	1.0
	0.02%	11.10 ± 0.41	49.2	2.5	26.2	4.2	17.1	4.1	7.5	3.7
	0.04%	10.23 ± 0.7	47.5	2.7	26.9	10.7	16.5	11.2	9.1	10.1
	0.06%	7.64 ± 0.52	40.7	5.7	33.8	25.1	15.7	16.5	9.8	14.5
	0.08%	5.12 ± 0.52	40.7	5.7	33.2	25.1	16.3	19.3	9.8	17.5
6h	Control	11.88 ± 0.21	44.2	0.7	27.1	0.9	17.5	0.9	11.2	0.7
	0.02%	10.9 ± 0.56	42.2	3.1	32.2	4.2	15.4	5.1	10.2	4.1
	0.04%	9.9 ± 0.11	41.7	4.9	30.3	11.1	14.9	10.9	13.1	4.1
	0.06%	7.8 ± 0.82	40.9	6.2	31.1	19.6	14.2	17.2	13.8	15.1
	0.08%	5.7 ± 1.3	38.3	7.1	34.7	26.5	15.5	22.1	11.5	18.4
9h	Control	10.6 ± 0.72	41.5	1.0	27.5	1.2	18.5	0.9	12.5	0.9
	0.02%	9.7± 0.61	41.1	4.2	33.7	4.9	12.1	4.3	13.1	4.3
	0.04%	9.5 ± 0.7	40.2	5.0	33.9	13.2	11.8	11.7	14.1	11.7
	0.06%	7.1 ± 0.66	38.7	6.4	35.1	27.5	13.1	17.2	13.1	17.2
	0.08%	6.3 ± 0.37	35.7	7.5	38.7	35.1	12.8	20.1	12.8	20.1
12h	Control	9.3 ± 0.61	40.1	1.3	25.7	1.4	20.1	1.2	14.1	1.0
	0.02%	8.7 ± 0.24	35.2	4.4	28.7	5.2	17.5	7.3	18.8	4.9
	0.04%	7.5 ± 0.31	32.1	6.1	33.4	15.7	17.1	15.3	17.4	13.7
	0.06%	4.1 ± 0.81	30.5	7.2	37.2	25.1	15.3	22.1	17.0	14.0
	0.08%	2.8 ± 0.46	29.7	7.9	39.7	35.4	10.2	29.9	20.4	22.5

Table 2.Effect of different concentrations of Tilt fungicide on the type and abnormalities (% abn.) of each stage in root tips of *Allium cepa*

Treatment		**Total**	**Types and percentage of abnormalities.**						
Time	Concentration	**%abn**	**CM.**	**Pr**	**St**	**Br**	**Lg**	**Mt**	**Ot**
	Control	2.6	0.0	0.0	0.0	0.5	0.0	2.0	0.0
	0.02%	4.1	49.2	2.0	1.1	0.9	0.0	2.1	0.0
3h	0.04%	13.5	2.1	0.0	2.3	2.9	1.7	2.7	1.8
	0.06%	19.9	5.0	0.0	3.1	3.0	1.8	4.8	1.4
	0.08%	25.2	6.0	1.1	3.9	4.1	2.2	5.7	2.2
	Control	2.7	0.0	0.0	0.3	0.4	0.0	0 2	0.0
	0.02%	2.6	0.0	0.0	0.0	0.5	0.0	2.1	0.0
6h	0.04%	5.6	0.0	0.0	1.3	0.9	0.7	2.3	0.4
	0.06%	17.2	3.3	0.0	3.1	2.3	2.3	4.7	1.5
	0.08%	25.3	7.2	1.8	3.1	2.7	3.1	5.3	2.1
	Control	2.8	0.0	0.0	2.0	0.5	0.0	0.3	0.0
	0.02%	7.1	2.4	0.0	1.7	0.7	0.0	2.3	0.0
9h	0.04%	22.9	5.6	2.3	2.1	1.8	3.1	6.0	2.0
	0.06%	27.7	7.7	2.7	2.7	2.3	3.0	7.1	2.2
	0.08%	35.1	9.9	4.5	3.4	2.9	3.4	7.7	3.3
	Control	2.2	0.0	0.0	2.0	0.2	0.0	0.0	0.0
	0.02%	14.7	2.6	1.1	3.1	2.4	0.0	3.7	1.8
12 h	0.04%	27.6	6.9	3.0	3.3	2.8	2.2	6.4	3.0
	0.06%	31.5	8.2	3.4	3.4	2.9	3.0	7.0	3.5
	0.08%	42.5	10.7	7.1	4.2	3.7	3.3	8.1	5.4

(Cm- C-

Metaphase, Pr-Precocious, St-stickiness, Br-bridge, Lg-laggard, Mt-multipolar, Ot-other)

Higher plants provide a useful genetic system for screening and monitoring environmental pollutants. The reduction in the mitotic index clearly indicates the mitodepressive and cytotoxic effects of the fungicide on the present test system. This might have been achieved by the inhibition of DNA [8].The presence of c-metaphase cells was on evidence of the action of pesticide concerned on the mitotic spindle [9].The sticky chromosomes have resulted in abnormal uncoiling of chromosomes during anaphase to telophase [10]. The cell division is energy dependent process and thereby the movement of chromosome mainly depends upon the energy generated system. The pesticide treatment disturbs the respiratory pathways resulting in low accumulation of energy containing and other essential compounds i,e ; ATP ,sugar and protein molecules' etc.The sticky nature of chromosome may be due to delay in chromosome movement by pesticide treatment. Thus the chromosome could not reach the poles and remained scattered in the cytoplasm and appeared condensed and sticky[11].Occurrence of diagonal spindle inactivation, which may be due to the inhibition of ATPase, by the interaction of pesticide on spindle proteins.[12] reported such spindle disorganizations in *Allium cepa*, were treated with pyrethroids. Bridges were found to be the result of stickiness of chromosome [13]. Another interesting abnormality noticed in the present study is the appearance of anaphase and telophase bridges involving one or more chromosomes. The bridge formation can be due to the general stickiness of the chromosome at metaphase stage, or breakage and reunion of chromosomes. Similar type of abnormality was also observed in the mitosis of *Vicia faba* after treatment with the organophosphorus insecticide [14]. Lagging chromosomes arise mainly due to abnormal spindle formation and as a results spindle fibre failed to carry the respective chromosomes to the Polar Regions and resultantly lagging chromosomes appeared [15].

CONCLUSION AND RECOMMENDATION

From these observations it can be concluded that the indiscriminate use of fungicide use in agriculture fields beyond their tolerance level can produce harmful effects on living systems including human beings.

ACKNOWLEDGEMENTS

Authors are thankful to the Head, P.G.Department of Botany, Vidya Bharati Mahavidyalaya Amravati, for the necessary facilities provided during the course of this work.

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