Journal of Advances and Scholarly Researches in Allied Education
Vol. 13, Issue No. 2, July-2017, ISSN 2230-7540

Genotoxic Effect of Culture Filtrate of Rhizoctonia Solani on Root Meristem Cells of Allium Cepa

Kumar Ajay[1]* Md Saifullah Fahim[2] Kumar Pramendra[3]

[1] HOD Department of Botany, Jagjiwan College, Ara

[2] Assistant Professor & Head Department of Botany AL- Hafeez College, Ara

[3] Part Time Faculty, H.D Jain College, Ara

Abstract – Genotoxic effect of 10 days old culture filtrate of a soil borne fungal pathogen i.e. Rhizoctonia salani were evaluated in root meristem cells of Allium cepa so as to assess the level of Genotoxicity. In the Allium root growth test the effective concentration i.e. 10 days old culture filtrate was determined significant. Mitotic index of the root meristem treated under culture filtrate was observed to be statistically significant after 120h treatment, marked genotype abnormalities i.e. stickiness, bridge, vagrant, multipolarity, c-Anaphase were also records during the investigation.

Keywords: - Allium Cepa, Culture Filtrate, Anaphase, Telophase, Chromosomes Aberrations, Genotoxic.

----------X----------

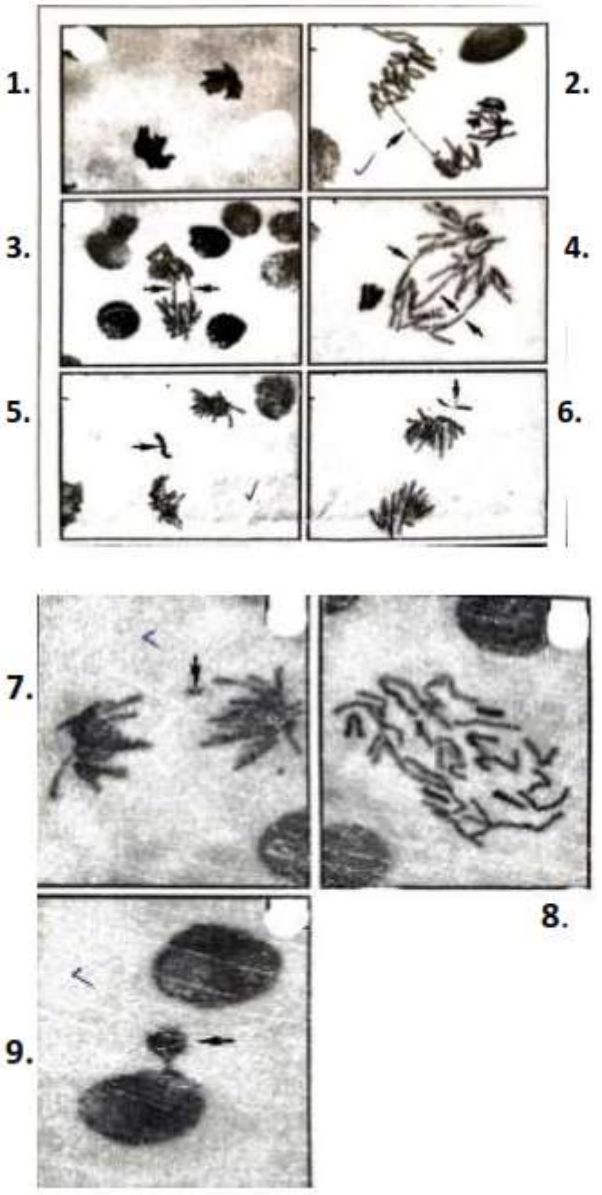
INTRODUCTION

Biological control measures are now being used in modern agricultural practices for obnoxious weed control. Higher plants provide valuable genotoxic assay system for screening & monitoring environmental pollutants. The *Allium cepa* is one of the frequently used higher plant species (Grant 1994).However, the *Allium cepa* test for genotoxity was introduced by Levan (1938) and has been used for pesticides in other studies Rank and Nielsen 1997, chauhan et. al., 1999, Chandra et. al. 2005. the Allium test was simple and reliable as the method in which chromosome aberrations were recorded in all types of mitotic cells. This test can used to measure both toxicity and genotoxicity. The rate of the root growth can be correlated with the mitotic index.

The chromosomes aberration & micronucleus assay have been shown to be highly reliable in genotoxicity testing (Natrajan 2002). the indiscriminate use of weedicide in agriculture ,as wellas the increase of pollution and biomagnifications , biological control or , vis-a-vis treatment is needed ,so culture filtrate of soil born pathogen like *R.solani* have evidence of inhibitory effect (Kumar Ajay,1990). So it is justified to assess the evaluation of the toxicity of these soil born fungal pathogen culture filtrate the purpose of this study was to investigate the effects of 10 day old culture filtrate of fungal pathogen Rizoctonia solani on root growth , mitotic index ,chromosome aberrations and micro nucleus formation in the root meristem cells of *Allium cepa*..

MATERIAL AND METHODS

Culture filtrate of *Rhizoctonia solani* was obtain after 10 days of their growth in sterile liquid Czapexdox nutrients medium and sterile distrilled water as well as czapexdox nutrient media served as control . Media contains (sucrose-30g, NaNo3=2g, kcl =0.59, kH2PO45H2O=1g,MgSo4=0.5g, Iron in traces & distilledwater1000ml).Fresh sterile bulb of Allium placed over the test tube for time limit of 4 days under aseptic conditions.The germinated root meristem treated with the culture filtrate was washed throughly and tip meristem of the emerged root was excised at 9am fixed in 1:3 Acetic Ethanol at every 24hrs of treatment . Trace of ferric choloride is added in the fixative as moderent . The root meristem of suitable size were squashed in acetocarmine and mitotic studies were made in each case after 6hrs of fixation. Mitotic index , chromosomes aberration , micronuclei were observed and counted by observing fixed no cells from freshly prepared slide in each case.



Photographic plate showing chromosomal aberration induced by cultural filtrate of R. solani after 120 hours.

1. Stickiness between chromosomes

2. , 3. , 4. – showing Bridges.

5. , 6. – showing vagrant-chromosomes

7. Showing anaphase stage with fragments. 8. Plate showing C- Anaphase.

9. Interphase cell showing Micronucleus

GENOTOXICITY TESTING OF 10 DAYS OLD CULTURE FILTRATE OF RHIZOCTONIA SOLANI IN ALLIUM CEPA ROOT MERISTEM AND MICRONUCLEUS ASSAY

Treatment Time(Hr)	Concentration	Mitotic Index do ± SE*	No. of cell examined	Anaphase-Telophase chromosome aberration					Micro-nucleus %
				Stickiness	Bridge	Vagrant	Anaphase	Fragment	
48	CF of R.solani	7.52	200	18	7	6	6	0	0.08
48	Medium as Control	22.20	200	0	1	0	1	0	0.00
48	Sterile distilled water as control	22.57	200	0	1	0	1	0	0.02
72	CF of R.solani	0.15	200.	23	10	10	8	1	0.09
72	Medium as Control	20.18		0	1	0	2	0	0.00
72	Sterile distilled water as control	12.52	200	0	0	0	1	0	0.00
96	CF of R. solani	5.12	200	25	16	13	12	1	0.02
96	Medium as Control	16.02	200	1	0	0	2	0	0.00
96	Sterile distilled water as control	10.12	200	0	0	0	0	0	0.0
120	CF of R. solani	2.19	120	27	18	16	13	2	0.21
120	Medium as Control	15.30	200	1	0	0	1	0	0.00
120	Sterile distilled water as control	8.52	200	0	0	0	0	0	0.00

RESULT

Effect on *Allium cepa* root growth and mitotic index. The root growth decrease with incresing hours of treatment of 10 days old culture filtrate of R.solani .Evidenced very less root growth after 96h of treatment. In both the controls the aeverage root was about 6.34+ o.18 cm was observed in both the control but more was dipicted in the medium control.

The effect of culture filtrate in the mitotic index(%) of *Allium cepa* root meristem Cell is determined in table 1 .Significant difference was observed in case of culture filtrate and the control ($P<0.05$). The mitotic index significantly decreaes compared to control at each exposer time. The percentage of mitotic index was significant after 120hour compared to 48,72,96 hour of treatment.

EFFECT ON CHROMOSOME AND MICRO NUCLEUS FORMATION

Results of genotoxicity test with the *Allium cepa* , anaphase, telophase, test where evaluated (table1) . the highest hour treatment of culture filtrate showed high toxicity on the root that is the root cells. Five type of chromosomes abberation in the anaphase , telophase, cells where observed which is shown in the photographic plate . Total 0/0 of stickiness ,bridges ,vagrant-chromosomes , c-anaphase and fragments .According to total cells evaluated with chromosomes aberration where calculated ,were found in increasing order with increasing hour of treatment. the stickiness was the most frequently not observed in both the control that is medium as control and distilled water control treatments. However the total chromosomes aberration increase with and increasing with the treatment hour. The total chromosomes aberration percentage where significantly higher at the highest hour of treatment.

Micro nucleated cells where observed at interphase shown in the photographic plate. The induction of micronucleus formation was generally observed in all different hour of treatment. However the micro

nucleus formation was significantly higher at 120 hours of treatment.

DISCUSSION

In the direction of biological control may be evaluated by analysing macroscopic (root growth decrease) as well as psychological parameters that is types and frequencies of chromosomes aberration (Kumar Ajay , 1990).

In Allium cepa root growth test culture filtrate was found toxic causing inhibition in root growth of *Allium cepa*. The decreases in root growth over 45% strongly indicates the presence of toxic substances having sub -lethal effect on plant(Hidalgo etal;1989).

The result of present study clearly indicates the utility of root meristem cells of *Allium cepa* in exploring a biological control or,vis- a -vis control majors .Increasing population of unwanted plants growing in the bear land areas , creating various health hazards to human being .However,the culture filtrate proved to be useful parameter for selecting Genotoxicity assays (MA .T.H et al. 1995,Chouhan et al. 1999).

Mitotic index is considered a parameter which allow to estimate the frequency of cellular division (Marcano et al 2004). Inhibition of mitotic activities is often used for tracing cytotoxic substances. The incresing hour of treatment is also depending inhibition of mitotic index illustrates the cytotoxic potential of culture filtrate of R. salani .Similar effects of mitotic index were also described by many reasearch scholar following treatment of with culture filtrate of *Fusarium udum* (Kumar Ajay et al.2015) among the chromosome aberrations observed stickness or sticky chromosomes as comman type ,where the most frequently observed abberation at anaphase , telophase stages of mitosis. Root tip cell of *Allium cepa* treated with culture filtrate is considered to be a chromatid type aberration (Darlington et al. 1951) suggested that stickiness has been shown to be results of DNA condensation (Osterberg et.al.1984) and interment of inter chomosomal fibres which lead to sub chromatic connection between chromosomes (Chouhan etal 1986) during present endavour the sticky chromosomes were observed at highers hour of treatment with high frequency as compared to controls .The bridge involving one or more chromosomes where most prominant and frequent type other than sticky chromosomes. The formation of bridge could be attributed to chromosomes breaks, stickness and reunion of the broken ends. The stickness of chromosomes prevented the separation of daughter chromosomes and remain connected by bridge (Bdar et.al1992) .sticky bridge may also be the result of incomplete replication of chromosomes by defective or less active replication enzyme(Sinha 1979).the spndle irreglarity like vagrant chromosomes c-anaphase were also obsorbed the induction of vagrant chromosomes leads to the unequal no of chromosomes in the daughter nuclei and subsiquently formation of daughter cells with unequal size at interphase described cholchocine mitosis ,c-metaphase or c-anaphase as an inactivity of the spindle (Levan 1938;El- Ghamery et .al 2003). Large numbers of vagrant chromosomes and c- anaphase indicate culture filtrate as a potent spindle inhibitor .on the other hand recording of micronuclei in interphase cell shows clastogenicity.

CONCLUSION

The test of chromosome aberration on plant system constitute a simple and reliable technique to detect the Genotoxicity of culture filtrate of soil born Fungal pathogen *R.solani*.

REFERENCES

Levan A. (1938) The effect of colchicine on root mitoses in Allium. Herididitas 24, pp. 471-486.

Sinha U., 1979- cytomorphological and macro molecular changes induced by P-flurophenylalanine in Allium cepa and Triticale .journal of cytologia and Genetics, 14: pp. 198.

Oesterberg R., Person D. and Bjursell G. 1984-The condensation of DNA by cromium(III)ions. Journal of Bimolecular structure and Dyanamics, 2: pp. 285- 290.

Hindalgo A., Gonzales -Rayes J.A, Noras P. and Garcia- Herdugo G., 1989 -Abnormal mitosis and growth inhibition in Allium cepa roots induced by Propham and chorpropham . cytobios,57: pp. 7-14.

Kumar Ajay and Mishra K B. 1990 – Cytological investigation on parthenium hysterophorus . L.affected by some Antibiotics andfungal filtrate Ph.D thesis ; M.U 1990.

Badr A. (1992) —cytotoxicity of some pesticides in mitotic cells of Vicia faba roots. Egyptian journal of Applied sciences 7: pp. 457 -468

Mishra K.B Kumar A. (1996). Effect of streptomycine on the growth of the seedling of Parthemium hysterophorus L. and mitotic division in the root. JBBS vol. 1&2; (pp. 114-117)

M A T.H. et. al. (1995)- the improved Allium /Vicia root tip micronucleus assay for clastogenicity of Environmental pollutants. Mutation research, 334; pp. 185-195

Natrajan A.T., 2002 – chromosomes aberrations: Past, Present and future .mutation research, 504: pp. 3-16.

Grant W.F., 1994 –the present status of higher plant bioaasay for the detection of environmental mutation. Mutation Research, 310; pp. 175-185.

EL Ghamery A.A., et. al. (2003)- Evaluation of cytological effects of zn2+ in relation to germination and root grown of Nigella sativa , and Triticum aestivum. L. Mutation research, 537: pp. 2941.

Chauhan L.K.S. (1999). Cytogenetic effect of Cypermethrin and Fenvalerate on the root meristems cells of Allium cepa .Environmental and Experinmental Botany, 94; pp. 181-189.

Marcano L., et. al. (2004). Cytotoxicity and mode of action of Maleic hydrazide in root tip. Of Allium cepa.L. Environmental Reasearch , 94; pp. 221-226.

Kumar Ajay, et .al (2015)- Genotoxic & Cytotoxic effects of culture filtrate of Fusarium udum on root meristem of Allium cepa – Scientia, vol. -3 ,issue -6, July – Dec.

Corresponding Author

Kumar Ajay*

HOD Department of Botany, Jagjiwan College, Ara