CYTOTOXIC EFFECT OF ETHYL ACETATE EXTRACT OF *PLEUROTUS* ON MITOTIC ACTIVITY IN *ALLIUM CEPA* L.

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ABSTRACT

Prolonged consumption of large quantities of mushrooms has led to toxicity, with toxins being the primary cause of most deaths related to mushroom poisoning. Consequently, the search for new active compounds from mushrooms has become crucial. In this study, ethanol extraction was performed on fresh fruiting bodies, and assays were conducted using commercially available onions. Observations were made from squash preparations with acetocarmine. The mitotic index, following treatment with varying concentrations of different *Pleurotus* extracts, showed a steady increase, except at higher concentrations of ethyl acetate. Chromosome stickiness during prophase was noted after treatment with concentrations. This stickiness was also observed at higher concentrations in *Pleurotus*. During metaphase, unoriented chromosomes frequently appeared after exposure to 1.5 and 2 percent of the fractions. Metaphase stickiness was induced to varying degrees at 1, 1.5, and 2 percent concentrations. Anaphase bridges were observed with higher concentrations of fractions. The study indicated that *Pleurotus* extracts exhibited some toxicity levels at comparable concentrations. It was concluded that prolonged and excessive use of *Pleurotus* might lead to chromosomal toxicity.

Keywords*: Pleurotus*, Cytotoxicity, Chromosome, Aberrations, Ethyl acetate, *Allium cepa* L.

INTRODUCTION

Mushrooms are observed during the rainy season on the manure heaps and dump places with abundance of humus. Mushrooms are better source of essential vitamins such as niacin, riboflavin and vitamin-C. They also contain folic acid, which is blood building vitamin and counteracts the pernicious anaemia, and is also highly rich in minerals such as calcium, phosphorus and potassium. Mushrooms grow in places like fields,woods, forests, water channels, manure heaps, bunds and grassy grounds. They can be easily grown under local conditions if proper requirements of food and humidity for its growth are fulfilled. Mushrooms

are cultivated on agricultural and industrial wastes and the wastes have to go through the process of boiling, pasteurization and fermentation. Straw, paper, saw dust, logs, rice, wheat straw are used as substrates. Although a great many species of mushrooms are edible, very few have been artifically cultivated. The most popular among these are European or White button mushroom (*Agaricus bisporus*), Paddy straw mushroom (*Volvariella spp*.), Oyster mushroom (*Pleurotus spp*.) and Shiitake mushroom (*Lentinus edodes*).

Plants and other living organismsare exposed to several chemicals that produce undesirable effects. A number of natural as well as processed materials are in our day to day use as food, drugs, cosmetics etc. without proper screening of their potential damage to human well being. Under certain conditions, plant products may induce mutagenic, genotoxic and cytotoxic effects, due to the presence of multiple biological properties. Human cells are continuously subjected to physiological and external influences which can give rise to cytotoxic, genotoxic and oxidative damage. However, cells have sophisticated mechanisms for counteracting and minimizing these types of damage. There are some mushrooms that contain exceptionally powerful toxins that represent a real hazard to health even when ingested in small doses. Most toxins were well studied and are described in literature, such as amatoxins that are cytotoxic and cause harm to kidney and liver and orellanine that is nephrotoxic (Karlson, 2003). Mushrooms were described as popular remedies in ancient oriental documents and some of them became ingredients in traditional medicine. Even in species with beneficial properties toxic substances were already found(Nieminen et al., 2006).Edible mushrooms are a valuable source of biologically active compounds. The use of mushroom with potential therapeutic properties raises global interest from scientific and clinical community based on their efficiency against numerous diseases and metabolic disturbances and natural, less expensive approach and in general involves minimal unwanted side effects. Recently, it was reported in France and Poland that several people developed delayed and, in some cases, fatal rhabdomyolysis after consuming large amounts of the yellow tricholoma (*Tricholoma equestre* or *Tricholoma flavovirens*) during several consecutive meals (Bedry et al., 2001). Phallotoxins and Amatoxins is responsible for most fatalities in mushroom poisonings. Fortunately, they occurs in only a few taxa.

In Kerala, commonly cultivated mushrooms are Oyster (*Pleurotus spp*.) and Milky *(Calocybe spp*.) types. Among these, oyster mushroom is the most widely cultivated type. Oyster mushroom cultivation is preferred due to low cost and high yield. *Pleurotus* is one of the edible mushrooms which can be cultivated in the tropics. It has gained importance only in the last decade and is now being cultivated in many countries in the subtropical and temperate zones.Several other species are now available for cultivation. These are *P. sajor- caju, P. florida* (probably a variant of *P. ostreatus*), *P. sapidus , P. eryngii* and *P.flabellatus* .

Pleurotus is a genus with a number of distinct species and strains. Popular in Japan and Central Europe. The oyster mushroom is so named because it looks similar to an oyster and is available in a wide array of colours, including white, yellow, brown, and pink, all tending to fade to a creamy gray when cooked.

The earliest record for *Pleurotus* cultivation in India appears to be that of first introduced the now popular tropical species.. It grows abundantly on the trunks of dead or dying deciduous trees. The most unusual location was on the dry skull of a dead whale reported in the 19th century.

MATERIALS AND METHODS

Fresh fruiting bodies of *Pleurotus was* obtained commercially,chopped into small pieces and dried, until constant weight is obtained. The dried fruiting bodies were powdered well using an electric grinder. 20g of powdered sample were extracted with 200ml of solvent ethyl acetate, Extraction was carried out in electric shaker with stirring at 150rpm for 8 hours at room temperature. The extract was filtered through whatman no 1 paper. The perfectly cleared solution was dried and stored. The dried powder obtained after extraction were weighed and a stock solution of 2 percent concentration was prepared. The stock solution was diluted to obtain the required test concentrations like 0.5, 1.0, 1.5 and 2 percent.

Onion culture

Approximately equal sized onion bulbs were bought from an open market. The bases of the bulbs were gently scrapped to expose the root primordia. To account for a number of bulbs in the population that would be naturally slow or poor growing, four replicate bulbs were used for each test sample and control (distilled water) and the best bulbs were chosen for test (Rank and Nielsen, 1997).

Cytotoxicity assay

For the evaluation of induction of chromosomal aberration, four onion bulbs were suspended on 0.5, 1, 1.5, 2% concentrations of ethyl acetate fractions and the control for 24 hours, at the end of which root tips from these bulbs were cut and fixed in Ethanol:Glacial Acetic Acid(3:1,v/v) for 24 hours.The fixed root tips were hydrolyzed in 1N HCl at 60°c for five minutes after which they were washed in distilled water. They were then squashed using aceto-carmine. The cover slips were sealed on the slides with clear fingernail polish as suggested by Grant (1982).this is to prevent drying out of the preparation by the heat of the microscope (Sharma,1983).Six slides were prepared for each concentration and the control. They were observed at ×100 magnification for induction of chromosomal aberrations. The mitotic index was calculated as the number of dividing cells per 1000 observed cells (Fiskesjo,1985 and 1997). The frequency of aberrant cells (%) was calculated based on the number of aberrant cells per total cells scored at concentration of each fraction (Bakare *et al.*, 2000).

RESULTS AND DISCUSSION

After treatment with different concentrations of ethyl actate extracts of *Pleurotus* on mitotic index in root tip cells of *Allium cepa L.*, indicated that a gradual increase is found, except in the case of higher concentration of ethyl acetate which posses a decrease in value.

Table 1

Effect of different concentrations of ethyl acetate extracts of *Pleurotus* on mitotic index in root tip cells of *Allium cepa L.*

Treatments	Mitotic index
Control	8.04
0.5	8.78
1	9.23
1.5	8.26
2	7.24

Graph1

Effect of different concentrations of ethyl acetate extracts of *Pleurotus* on mitotic index in root tip cells of *Allium cepa*

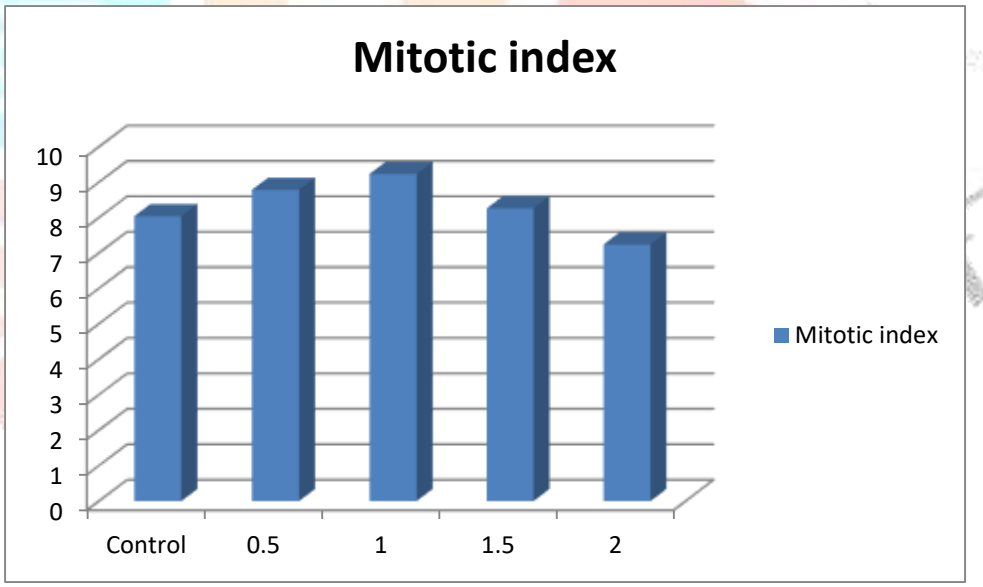

Table2

Index of various mitotic stages after treatment with ethyl acetate fractionof *Pleurotus* on mitosis in root tip cells of *Allium cepa* L.

Treatments	Prophase	Metaphase	Anaphase	Telophase
Control	56.47	14.29	17	12.24
0.5	55.28	11.67	25.28	7.28
1	47.91	24.56	16.29	12
1.5	39.28	27.28	19.25	14.21

2	40.54	24.3	20.71	14.48

Graph2

Index of various mitotic stages after treatment with ethyl acetate fractionof *Pleurotus* on mitosis in root tip cells of *Allium cepa*L

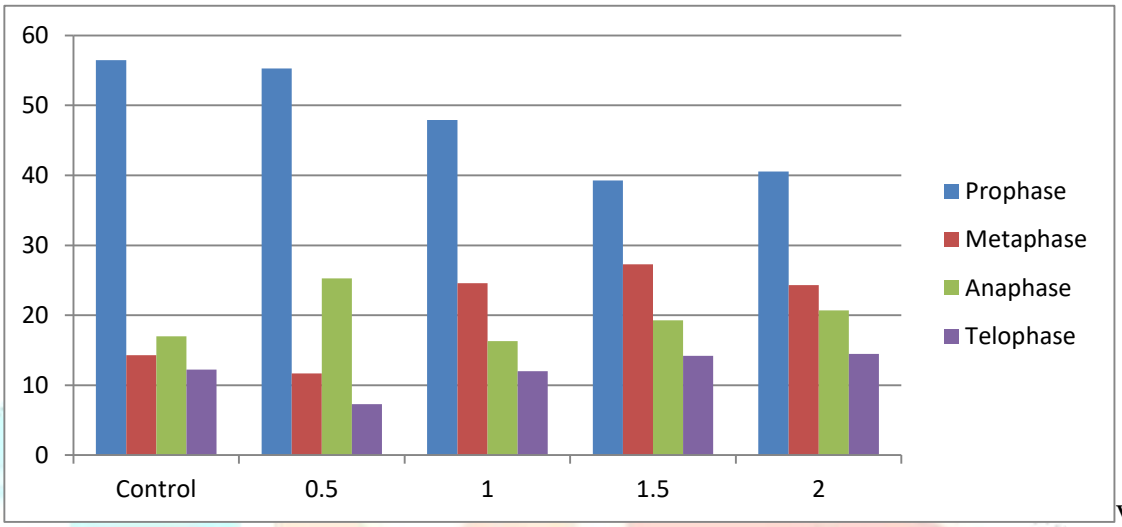

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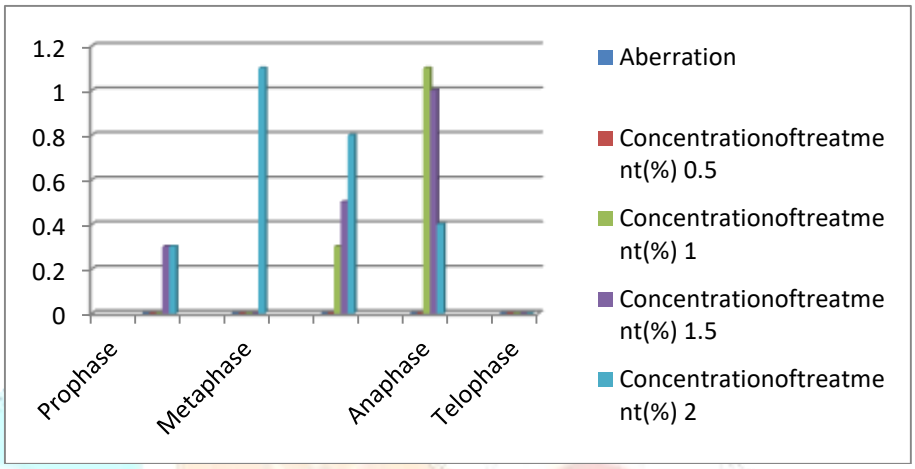
Table-3

Percentage of abnormalities met within the meristematic cells after treatment with various fractions of *Pleurotus* on root tip cells of *Allium cepa L.*

Stage	**Aberration**	Concentration of treatment(%)			
		0.5	**1**	**1.5**	**2**
Prophase	Stickiness	0	0	0.3	0.3
Metaphase	Unoriented	0	0	0	1.1
	Stickiness	0	0.3	0.5	0.8
Anaphase	Bridge	0	1.1	1	0.4
Telophase	Bridge	0	0	0	0

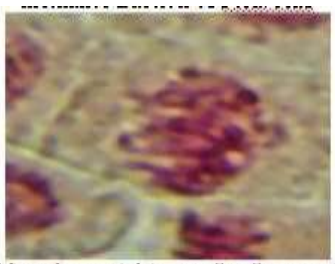
Graph-3

Percentage of abnormalities met within the meristematic cells after treatment with various fractions of *Pleurotus* on root tip cells of *Allium cepa L.*

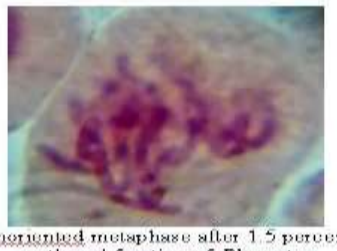


From Table 3 ,it was found that stickiness of prophase was observed after treatment with higher concentrations. In the case of metaphase, un-oriented chromosomes are of frequent occurrence after 2 percent of the fractions. In the case of metaphase, 1, 1.5 and 2 percent concentration induces varying degrees of stickiness. Bridge at anaphase was observed at treatment with higher concentration of the fractions.

The *Allium cepa* assayis an efficient test for chemical screening and *in situ* monitoring for genotoxicity of environmental contaminants. The onion genotoxicity test provides for easy screening of



Metaphase stickiness after 2 percent ethyl acetate fraction of *Pleurotus*



Unoriented metaphase after 1.5 percent methanol fraction of *Pleurotus*

chemicals or samples with genotoxic effects, especially to plants. (Freiti *et al.*, 2007).The abnormalities observed with respect to treatment with various fractions of *Pleurotus* indicated that stickiness is predominating. Stickiness is the major abnormality obtained in the present study. Stickiness is due to inter chromosomal linkages of sub-chromatid strands coupled with excessive formation of nucleoproteins and inappropriate protein-protein interaction (Badr and Ibrahim, 1987). The latter is also believed to have resulted from altered physico-chemical properties of DNA due to interactions with other chemicals like mutagens, carcinogens and clastogenic agents (Badr and Ibrahim, 1987). A very low frequency of bridges are also observed along with stickiness among theaberrant cells. The presence of dicentric chromosomes and unequally exchanged chromatids undergoing translocation has been reported to be responsible for chromosomal bridges at anaphase (Badr and Ibrahim, 1987). Un-orientation of metaphase chromosomes was also found to occur among the treated population. Un-orientation at metaphase and scattering of chromosomes may be due to either the inhibition of spindle formation or the destruction of spindle fibres formed (Kumar and Rai, 2007).

CONCLUSION

The study investigated the cytotoxic effects of ethyl acetate extracts of *Pleurotus* mushroom on root tip cells of *Allium cepa* L. (onion). Different concentrations of the extracts (0.5%, 1%, 1.5%, and 2%) were tested. The mitotic index showed a gradual increase with increasing concentrations, except for the highest concentration (2%), which showed a decrease. Various mitotic stages were affected differently by the treatments. Chromosomal aberrations such as stickiness, unoriented chromosomes, and bridges were observed at higher concentrations. Stickiness was the predominant abnormality, possibly due to altered physico-chemical properties of DNA caused by interactions with mutagens, carcinogens, or clastogenic agents. The *Allium cepa* assay proved to be an efficient test for screening the genotoxicity of environmental contaminants, especially in plants. It was concluded from the present study that the use of *Pleurotus* for a long run and greater quantity may induce a chance of toxicity at chromosomal level.

REFERENCES

Abdou RF, Megalla SE, Moharram AM, Abdel-Gawad KM, Sherif TH, el-Syed Mahmood AL, Lottfy AE. 1989. Cytological effects of fungal metabolites produced by fungi isolated from Egyptian poultry feedstuffs. *J. Basic Microbiol.* 29(3):131-9.

Badr, A. and A.G. Ibrahim, 1987. Effect of herbicideGlean on mitosis, chromosomes and nucleic acids in *Alium cepa* and *Vicia faba* root meristems. Cytol., 52: 293-302.

Bakare AA, Mosuro AA, Osibanjo O. 2000.Effect of simulated leachate on chromosome andmitosisinrootsof *Allium cepaL.J.Environ. Biol.* 21(3): 263-271.

BedryR ,BaudrimontI, Deffieux G, CreppyEE,PomiesJP,RagnaudJM, DuponM,NeauD,GabinskiC,DeWitteS,ChapalainJC,GodeauP.2001. Wild-mushroom intoxication as a cause of rhabdomyolysis. *New Engl J Med 345*:*798*–802.

Bilgrami,K.S.JammaludinandRizvi,M.A.1979.Fungi of India.Part-II. Today & Tomorrow's Printers and Publisher, New Delhi.

Feretti, D., Zerbini, I. and Zani, C .2007: *Allium cepa* chromosomeabberation andmicronucleustestsappliedtostudygenotoxicityofextractsfrompesticide-treated vegetables and grapes. *Food Addit. Contam.* 24 (6): 561- 572.

Fiskesjo,G.,1985.The*Allium*testasstandardin environmental monitoring.Hereditas,102:99-112.

Fiskesjo,G., 1997. *Allium* test for screening chemicals: Evaluation of cytologic parameters. In**:** Plants for Environmental Studies, Wang, W., Gorsuch, J.W., Hughes, J.S. (eds), CRC Lewis Publishers, BocaRaton, New York. 308-333.

Grant,W.F.1982.Chromosome aberrationassays in*Allium.*A report of the United States Environmental Protection Agency Gene Toxicity Program. *Mutation Research*, 99:273-291.

Karlson-StiberandPersson.2003.Cytotoxicfungi–anoverview.*Toxicon.*42(4):339-49.

Kumar, G., and Rai, P.K., 2007. EMS induced karyomorphological variations in Maize (*Zeamays* L.) inbreds. Turk J Biol, 31: 187-195.

NieminenP,Kirsi.MandMustonenA.M.2006.SuspectedMyotoxicity of edible wild mushrooms. *Experimental Biology andMedicine* 2006; 231: 221-8.

Rank J. 2003. The method of *Allium* anaphase-telophase chromosome aberration assay. *Ekologija* 1: 38-42.

Rank, J., Nielsen, M.H. 1997. *Allium* anaphase-telophase genotoxicity assay. Department ofEnvironment, Technology and Social Studies, Roskilde University, Denmark.

Sharma, S.K., 1983. New species of ceraphonoidea from India (Hymenoptera) Entomotaxonomia 5(3):19-224.