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# Study of Some Cytotoxic Chemicals on Onion (*Allium cepa* L.) and Grass pea (*Lathyrus sativus* L.)

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**Abstract**

Chemicals that have adverse effects on normal cell division are called cytotoxic chemicals. Root Length, mitotic index and cytological studies of root tips in the presence of different cytotoxic agents were experimented on Onion (*Allium cepa* L.) and Grass pea (*Lathyrus sativus* L.). Different chromosomal abnormalities were observed from the root tip cytology. The root Length decreases most in higher concentration of the chemical with respect to control. The most frequent abnormalities are bridges, vagrant and stickiness of chromosome. Grass pea shows two additional abnormalities of pyknosis and karyorrhexis. The results show that the chemicals have toxic effects on both the samples. This study proves that besides onion, grass pea can also be used as effectively for cyto-genotoxic assessments for environmental toxic agents.

## INTRODUCTION

Use of insecticides is a common pest control process for household and agriculture purpose (Sharma, 2011). Bavistin is one of the most used fungicides especially in third world countries (Mishra, 1988, Sakhale and Kapse 2012). Cypermethrin is a pyrethroid insecticide which has several cytotoxic effects on insects, pests and human beings (World Health Organization 1989, Das 2006 and Parajuli, Chakravarti *et al*. 2007, Sharma 2011). Gamaxine is a common insecticides used for household purposes (Li YF 1999, Vijgen *et al*. 2011). These chemicals are available in the open market in the form of dust. This study has been done to assess the cytogenotoxic effects of some common insecticide and pesticide on Onion (*Allium cepa* L.) and grass pea (*Lathyrus sativus* L.). Onion is recommended as a model plants for cytogenetic study by United States environmental protection Agency (USEPA) and American Society for Testing and Materials (ASTM) (Fiskesj 1985 a, Fiskeji1985 b, Fiskesj 1997). This plant is also used as water pollution indicator due to the presence of some activating enzymes in root tip cells (Grant 1982, Rank and Nielsen 1997, Rank 2003, Babatunde and Bakare 2006). They are used as a model for testing of genotoxic materials. They are chosen for easy preparation and distinct observation of chromosomes (Bakare *et al*. 2009, Olorunfemi and Ehwre 2010).Grass pea is chosen as it is easily available in south-west Bengal and whether they can be used as an alternative model for cytogenotoxic study. This plant has been chosen as a model for studying cytological effects of

anticancerous drugs (Samanta *et al*. 2015). The present study is done to assess the cytogenotoxic effects of a pesticide (Gamaxine), insecticide (cypermethrin) and a fungicide (Bavistin) on grass pea so as to use it as an alternative model for genotoxic tests.

## MATERIAL AND METHODS

Onion bulbs (*Allium cepa* L. [2n=16]) and seeds of grass pea (*Lathyrus sativus* L. [2n=14]) were bought from local market of Contai, West Bengal. The bases of the bulbs were shaved off with a razor blade to expose fresh meristematic tissue and then dipped into 60ml culture test tubers containing for 72 hours (3 days).The grass pea seeds were placed to a cotton bed soaked with different concentration of test materials as mentioned by Samanta *et al.* (2014). The sets of bulbs and seeds (15 seeds in each set) were made in triplicates.The root length (in mm) was measured with a scale millimetre graph attach with a slide. The main root length of three bulbs of each sample was taken and statistically recorded.

### Methods

After 72 hours, root tips from the treated bulbs and seeds were cut and kept in acetic acid and alcohol (1:3) solution. This solution acts as a fixative for the specimen. After 3 hours the root tips were taken and placed into watch glass containing 2% Acetorcein and 1(N) HCl (9:1) solution for staining the tips with the staining solution was gently heated by spirit fame for 3 minutes. After 45 minutes the specimen was taken in to slide, the extreme tip portion cut by a blade.Excess stains washed by 45% acetic acid and covered by coverslip. A gentle tipping and pressure were applied around the round area of the coverslip for squashing of the specimen. The edges of the coverslip aresealed by nail hardener to prevent drying out (Sharma 1983). The slides were observed under the compound microscope at X1000 magnification to observe mitotic stages and chromosomal abnormality.

### Statistical Analysis

The mean (95% confidence) and standard errors for results of root-shoot length and chromosome aberrations were calculated. Expression of data was as Mean standard Error of Mean (SEM).The significant values considered in $p<0.05$.

## RESULTS AND DISCUSSION

The mean root length and mitotic index of the two plant specimens were decreased with an increase of the concentrations with respect to control **(Table 1)**. In case of onion the root length decreases most for the Bavistin (84.08%) than for Cypermethrin (79.05%) and Gamaxine (64.53%). Mitotic index decreases most for Gamaxine (83.67%) than for Cypermethrin (79.54%) and Bavistin (71.81%). On the other hand the root length of grass pea decreases most for Gamaxine (89.48%) than for Cypermethrin (81.97%) and Bavistin (79.90%). The decrease of Mitotic indexis most for Gamaxine (98.08%) than for Bavistin (92.42%) and Cypermethrin (88.01%).

The value of the mitotic index below 22% as a companion to control causes lethal effects on an organism (Antonise-Wiez, 1990). Both the specimen shows the decrease in root length and mitotic index though in different manner for different chemicals. The reduction of mitotic index signifies the toxic effect of the test materials on cell division of selected plant specimens. All the results of reduction of root-shoot length and mitotic index as compared to control is statistically significant ($p<0.05$).

**Table: 1: The mean root length and mitotic index of onion and grass pea in presence different concentration of cytotoxic chemicals.**

| Plant name | Concentration (%) of Cypermetherin | Mean Root length(cm)±Standard error | Mitotic Index |
|---|---|---|---|
| Onion | 0 | 5.78±0.78 | 15.007 |
| | 0.02 | 3.76±0.24 | 10.37 |
| | 0.04 | 2.91±0.16 | 5.68 |
| | 0.06 | 2.07±0.24 | 4.21 |
| | 0.08 | 1.21±0.08 | 3.07 |
| Grass Pea | 0 | 10.65±0.94 | 18.752 |
| | 0.02 | 5.19±0.21 | 13.56 |
| | 0.04 | 4.38±0.34 | 7.61 |
| | 0.06 | 3.23±0.24 | 3.94 |
| | 0.08 | 1.92±0.13 | 2.25 |
| **Plant name** | **Concentration (%) of Gamaxine** | **Mean Root length(cm)±Standard error** | **Mitotic Index** |
| Onion | 0 | 5.78±0.78 | 15.007 |
| | 0.02 | 4.34±0.42 | 8.52 |
| | 0.04 | 3.48±0.23 | 5.41 |
| | 0.06 | 2.28±0.19 | 3.27 |
| | 0.08 | 2.05±0.17 | 2.45 |
| Grass Pea | 0 | 10.65±0.94 | 18.752 |
| | 0.02 | 7.57±0.36 | 4.56 |
| | 0.04 | 4.21±0.25 | 3.59 |
| | 0.06 | 2.24±0.18 | 1.73 |
| | 0.08 | 1.12±0.13 | 0.36 |
| **Plant name** | **Concentration (%) of Bavistin** | **Mean Root length(cm)±Standard error** | **Mitotic Index** |
| Onion | 0 | 5.78±0.78 | 15.007 |
| | 0.02 | 2.26±0.35 | 13.88 |
| | 0.04 | 2.24±0.31 | 12.45 |
| | 0.06 | 1.28±0.29 | 10.88 |
| | 0.08 | 0.92±0.06 | 4.23 |
| Grass Pea | 0 | 10.65±0.94 | 18.752 |
| | 0.02 | 8.33±0.56 | 5.69 |
| | 0.04 | 5.04±0.41 | 4.16 |
| | 0.06 | 4.12±0.23 | 3.32 |
| | 0.08 | 2.14±0.16 | 1.42 |

The aberration of chromosomes were observed in all concentrations for both onion and grass pea (**Fig 1**). The mostly present abnormalities are bridges, stickiness, clumping, and fragments. The decreased root length shows cytotoxicity and chromosomal aberration signifies the geno-toxicity of test materials. The frequent presence of sticky chromosome, at metaphase and Anaphase Bridge, is the indication of mutagenic activity (Mishra, 1993). The sticky surface of a chromosome is the indication of cell death (Fiskesj 1985 a). For grass pea, theaddition to the above-mentioned abnormalities other

nuclear abnormalities were found. One is pyknosis (nuclear shrinkage) and other is karyorrhexis (nuclear fragmentation). These are the indication of cellular necrosis (Bindu *et al*. 2003).

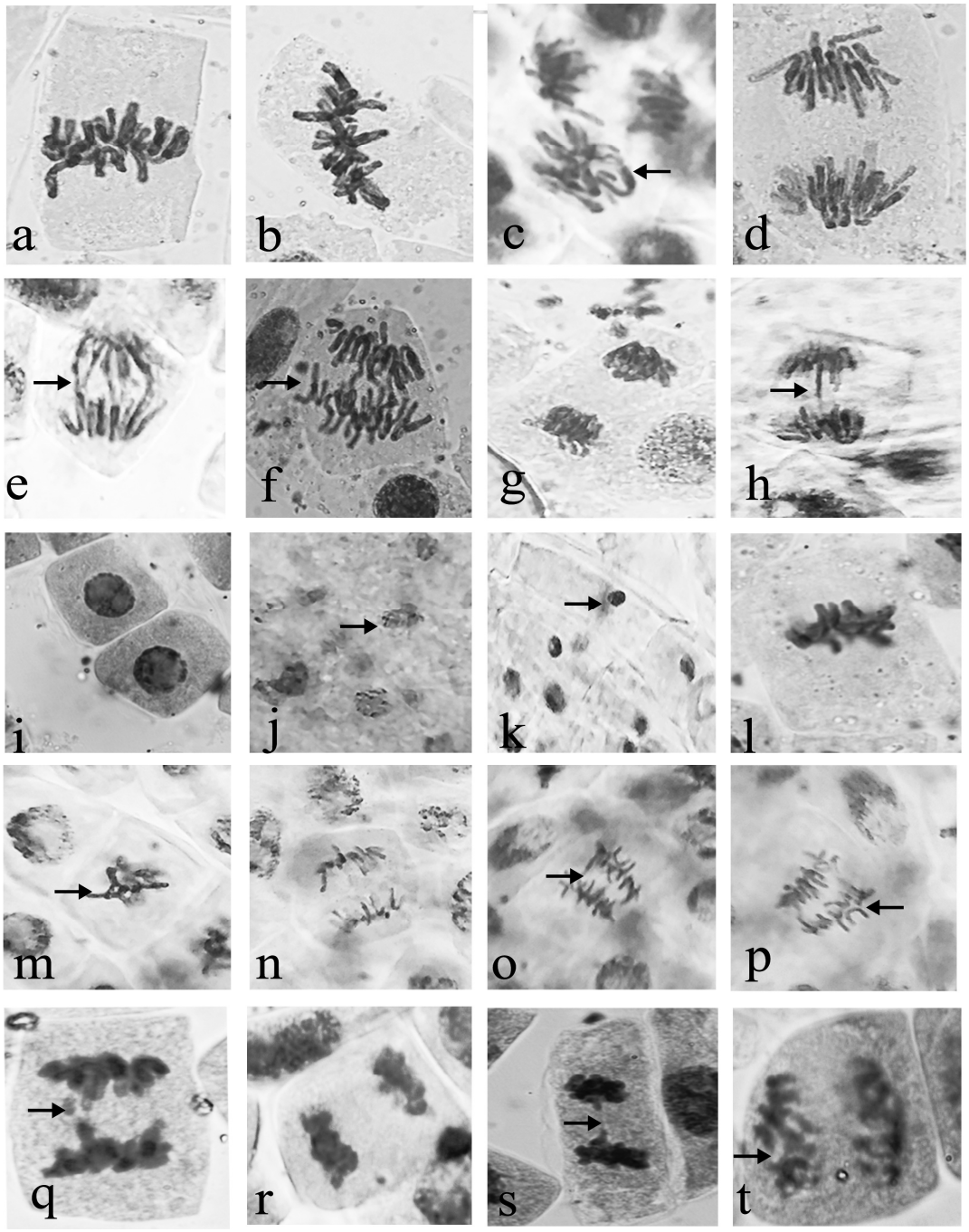


**Figure 1: Different chromosomal abnormality as observed in Onion (a.) Normal metaphase, (b.) Sticky metaphase, (c.) Metaphase clumping, (d.) Normal anaphase, (e.) Anaphasebridge, (f.) Abnormal separation (g.) Normal telophase, (h). Telophase bridge) and Grass pea (i.) Normal interphase, (j.) Nuclear fragmentation, (k.) Nuclear shrinkage, (l.) Normal metaphase, (m.) Sticky metaphase, (n). Normal Anaphase, (o.) Anaphase bridge, (p.) Vagrant chromosomes, (q.) Acentric chromosome, (r.) Normal telophase, (s.) Conjoined telophase, (t.) Multipolarity) root tip cytology.**

## CONCLUSION

*Allium cepa* L. is generally used to study different genotoxic effects on plants. *Lathyrus sativus* L. also shows similar types of cytotoxic effects with some additional abnormalities. In the study; it is proved that grass pea could also be an effective tool for monitoring cyto-genotoxic effects of toxic chemicals.

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