Poll Res. 40 (3) : 777-781 (2021)

ISSN 0257–8050

EFFECT OF PRESERVATIVES AND PESTICIDES ON MITOTIC INDEX OF *ALLIUM CEPA* ROOTS -BIOLOGICAL MODEL EXPERIMENT FOR GENOTOXICITY

JYOTCHNA GOGOI[1], KARABI DAS[2] AND PAPIA DUTTA[3]

[1,2]Faculty of Sciences (Biochemistry), Assam Downtown University, Panikhaiti 781 026, Assam, India
[3]Faculty of Sciences (Chemistry), Assam Downtown University, Panikhaiti 781 026, Assam, India

(Received 18 December, 2020; Accepted 17 January, 2021)

ABSTRACT

In research, Genotoxicity model: *Allium cepa* has often been used due to its cost effectiveness and basic laboratory requirements. Many studies based on mitotic index in *Allium cepa* roots have been performed to observe toxicity of various contaminants. So, a study was designed to conduct genotoxic evaluation in *Allium cepa* roots caused by food preservatives and pesticides which could be used in college experiments with minimum health concern. Microscopic observation like Chromosomal aberrations and mitotic index was documented by Foldscope. *Allium cepa* bulbs were allowed to grow under different treatments- normal distilled water (treatment 1), malathion (treatment 2), chlorpyrifos (treatment 3), residual pesticides (treatment 4), monosodium glutamate (treatment 5), sodium benzoate (treatment 6) and propyl gallate (treatment 7) with an optimal dose concentration (100 µg/ml) with an interval of 24 hrs. Mitotic index of the dividing cells were calculated for duration of 24 hrs to 72 hrs. The results showed highest mitotic index in normal (treatment 1): 63±0.8 and 68±1.1, whereas treatment 5 showed lowest mitotic index of 22±0.46 and 15±1.02 in 24 hrs and 72 hrs, respectively. Thus, it could be concluded that food preservatives Monosodium glutamate can be used as standard for the experiment in college which would help the students to understand genotoxicity at ease with minimum use of sophisticated instruments.

KEY WORDS : Genotoxicity, *Allium cepa*, Chromosomal Aberrations, Mitotic Index, Foldscope

INTRODUCTION

Genotoxicity is the ability of toxic components to damage the genetic information within a cell. Such damages can cause heritable changes. Several *in vitro* studies are done to assess the potentiality of such toxic compounds to sustain the safety of the environment. Various pesticides such as insecticides, herbicides, fungicides, etc. are extensively used in agricultural fields which adversely effect human health and the environment. Acute pesticide poisoning is known to cause mortality worldwide, especially in developing countries like India (Abhishek *et al*., 2014). *Allium cepa* is used as a potential biomarker of genotoxic studies as they help to study toxic compounds that decrease their mitotic index (Cabuga *et al*., 2017). Experiments conducted using *Allium cepa* model is reported to be helpful in studying the toxicity of various food preservatives, pesticides, contaminants in drinking water, azo dyes etc. The mechanism involved in the test is observing the mitotic index of the cells where a gradually decreasing mitotic index value indicates the toxicity of the contaminants upon division of these cells. Preservatives are reported to cause chromosomal aberrations thereby decreasing the mitotic index with increase in dose concentration in longer treatment times (Pandey *et al*., 2014). Studies have also revealed the capacity of pesticides in decreasing cell viability and their capability of producing genetic effects including cancer and other pathologies in humans (Bolognesi *et al.*, 2011). Toxicity evaluation of such preservatives and pesticides using *Allium cepa* has been the most

efficient since, similar toxic effects are observed in human lymphocytes (Grover *et al.,* 1990). Such experiments prove to be excellent as here the roots grown are directly exposed to the contaminant which enables damage to the DNA. Environmental agencies like the United Nations Environmental Program, World Health Organization, International Program on Plant Bioassay and US Environmental Protection Agency have advocated and validated the use of plants as test organisms (Yuzbasioglu *et al*., 2009). The main component of the *Allium cepa* test system is a vascular plant, hence considered to be advantageous and serves as an excellent genetic model in evaluating environmental pollutants, detecting mutagens in environments, etc.

The above study was designed to observe genotoxicity of food preservatives and pesticides using *Allium cepa* as a test model and the feasibility of this experiment to be conducted in colleges. Such experiments require very less instruments and are not restricted to well establish high tech labs. Many Govt. and even private colleges are not well equipped enough to conduct many experiments that are of global concern. In such a scenario, being able to conduct experiments related to toxicity of food preservatives and pesticides in college level can be of immense significance. For this, pesticide's and preservative's toxicity were observed using *Allium cepa* and visualization of chromosomal aberrations was facilitated by foldscope (*a paper microscope*) (*www.foldscope.com*). It's low cost, portability and durability makes it an effective tool for studying and visualizing genotoxicity of cells, especially for biology students (Das *et al.,* 2019). With minimal use of instruments and avoiding complex experimental set ups, these types of studies can enhance the interests of students in learning biology.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents used in the present study were of analytical grade (*Merck and HiMedia*). Pesticide standard - Malathion, Chlorpyrifos *(PESTANAL®)* and preservatives - Monosodium glutamate (MSG), Sodium benzoate (SB), Propyl gallate (PG) was purchased from *Sigma- Aldrich*. *Aceto orcein* stain was used for staining the root tips.

Extraction of residual pesticides

Fruits were collected from local markets of Guwahati, Kamrup (Metro) District of Assam. A total of 47 samples were collected during the month of June-September 2018. The fruits so collected were thoroughly washed and stored in deep freeze (-4 ºC) for its freshness until analysis. Prior to analysis they were kept in room temperature (H"25 ºC), weighed and cut into small pieces manually. The solvent used for the extraction of residual pesticides was a mixture used of toluene, hexane and ethyl acetate in the 3:1:1 (Choudhary and Bhatnagar, 2016). The samples were infused in the extraction mixture for an hour and then filtered with Whatman filter paper. The filtrate so obtained was heated at 30 ºC to evaporate the solvent and obtain dried residual pesticide extract. Triplicate sets (*n=3*) of extraction were done. The dried residual extract was further used for the study.

In vitro genotoxicity study

Food preservatives, pesticide and extracted residual pesticide samples were assayed for genotoxicity study in *Allium cepa* root tips (Matsumoto *et al.,* 2006). Fresh *A. cepa* bulbs were cleaned, and outer scales were removed leaving the primordial roots intact. The bulbs were kept suspended in 15 ml glass vials leaving the roots in contact with distilled water. The bulbs were maintained at photoperiod (13h/11h light/dark) and temperature (25 ºC±3 ºC). Bulbs with 2cm long roots were used for the study (Viel *et al*., 2019). After, growth of root tips, toxicity study was done for 3 days. The bulbs were divided into seven groups (Table 1).

Seven groups classified were as follows - Group1 (treated with distilled water) served as Control, Group 2 (Malathion Std. treated), Group 3 (Chlorpyrifos Std. treated), Group 4 (residual pesticide treated), Group 5 (MSG treated), Group 6 (SB treated) and Group 7 (PG treated). Among these, chlorpyrifos, MSG, SB and PG stock solutions were prepared in distilled water. Malathion and extracted residual pesticide stock solution were prepared by dilution of 1000 µg/ml in methanol. Further working solutions of different concentration, i.e. 50µg/ml, 100 µg/ml and 200 µg/ml were used for the study. The roots of treated and control bulbs were cut and fixed in ethanol: glacial acetic acid (3:1, v/v) for 20 min. These were hydrolyzed in 1N HCl (Hydrochloric acid) at room temperature for 45 min, after which they were rinsed in distilled water. The roots were stained with Aceto orcein. Microscopic cellular changes were documented by using Foldscope (140X magnification) for seven days. For better visualization and capturing microscopic

Table 1. Table showing classification of onion bulbs for different treatments in varying dilutions

Group	Treatment		Dose concentration (µg/ml)		
1	Control	Distilled water	50	100	200
2	Pesticide	Malathion Std.	50	100	200
3		Chlorpyrifos Std.	50	100	200
4		Residual pesticide	50	100	200
5	Preservative	MSG	50	100	200
6		SB	50	100	200
7		PG	50	100	200

images Foldscope was coupled with camera lens of mobile phone using magnetic couplers (Foldscope, DBT, India and Nokia 7 plus India).

Discarding the *Allium cepa* bulbs

Allium cepa bulbs used for the above study were treated with different pesticides and preservatives which are capable of imposing serious threats to the environment. Therefore, after completion of the study the bulbs need to be discarded off properly which would otherwise turn out to be hazardous. Hence, all the *Allium cepa* bulbs were burnt completely into ashes. Burning would convert these contaminants into their elemental state so that they do not build up in the environment again.

RESULTS AND DISCUSSION

The extracted residual pesticides were white, flaky, and amorphous in nature. Dose (50 µg/ml, 100 µg/ml and 200 µg/ml) study was performed and each day the cellular changes were documented using foldscope. Images were captured using magnetic couplers and camera lens of Nokia 7 plus mobile phone. Among the three doses, 100 µg/ml of concentration was found to provide the best results and hence the mitotic index values of 100 µg/ml concentration of dose are depicted in Table 2. During the study (three days), normal division of cells was observed in Group 1 (distilled water treated) tips (*A. cepa*) with a mitotic index of 63±0.8 and 68±1.1 in 24 hours and 72 hours respectively. Several mitotic stages such as metaphase, anaphase, late anaphase, telophase, prophase (Figure 1: A-B) were seen under foldscope. Whereas changes in cell size and shapes along with chromosomal aberrations were observed in the other groups. The mitotic index as calculated is shown in Table 2. In all the other treatments (2-7) cellular damages were observed from day 1 to severe chromosomal aberrations in day 3. Further toxicity study couldn't be conducted as tips were completely damaged after day 3. However, root tips of the control set treated with distilled water were healthy and fresh Chromosomal aberrations such as irregular metaphase, micronucleus formation, abnormal cell shapes and *sizes* and cell necrosis (Fig. 2. C-H) were observed in the treated bulbs (except the control set). The severity of toxicity as observed was found to be in the following order: residual pesticide< chlorpyrifos<malathion in case of pesticides and SB<PG<MSG in case of preservatives.

Toxic effects of pesticides and preservatives are a serious matter of global concern. Unlimited use of pesticides and preservatives causes severe health issues. They cause adverse effect to environment such as pollution and causes imbalance of

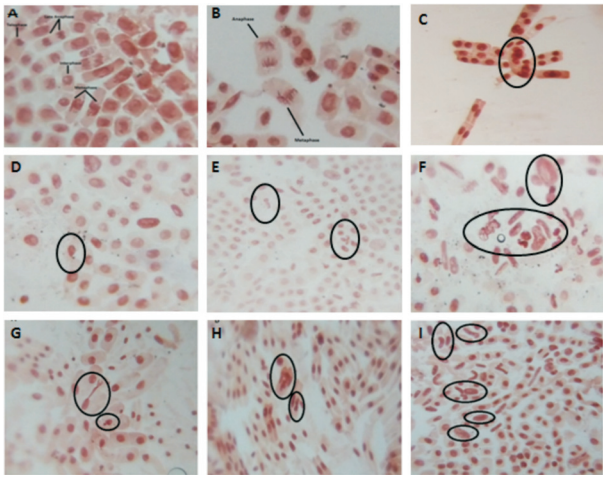

Fig. 1. Normal mitotic stages in untreated onion roots (A-B); abnormal division patterns in different treatments (C-H). (A) Late anaphase and metaphase; (B) Anaphase and metaphase; (C) Stickiness of cells (D) Micronucleus formation (E-F) Abnormal cell patterns and shapes; (G) Micronucleus formation; (H) Abnormal metaphase; (I) Abnormally enlarged nucleus and irregular division patterns of cells. *Images taken under Foldscope with 140X magnification lens using Nokia 7 Plus mobile handset with 2X optical zoom

ecosystem. Even in less concentration they are capable of affecting the environment (Mahmood *et al*., 2015). In the above study, the dose concentration of 100 µg/ml is found to be effective and showed considerable chromosomal aberrations. Our results agree with the findings of Turgoklu (2007), which states that preservatives significantly decrease the mitotic index value of onion root tips compared to the control at different time periods (Turkoglu, 2007). The presence of stickiness (Figure 1C) in our result supports the view of Pandey *et al.* (2014), who states that this stickiness of cells indicates an effect on proteins of chromosomes which was found on treatment with propyl gallate. There are studies which state that sodium benzoate increases formation of micronucleus under different concentrations and intervals of time (24 and 48 hour) (Patel and Ramani, 2017). In the above study, among the pesticides Malathion and among preservatives MSG was found to be the most toxic. Malathion is reported to have serious effects on human health, aquatic life and the environment (US EPA). On the other hand, MSG which serves as a flavoring agent is the most extensively used food additive. It has its deleterious effects on central nervous system, reproductive organs, hepatic tissues, adipose tissues, etc (Husarova and Ostanikova, 2013). According to the EFSA (Europe's Food Safety Authority), permissible amount of MSG is 30 mg per kilo body weight (bw) per day (Food Navigator.com). This safety intake level is based on toxicity studies in test animals exceeding which would adversely affect them. Monitoring, if MSG is used in foods under the mentioned permissible amount would be a study of immense importance.

The chromosomal abnormalities observed under foldscope in treatment 4 prove the genotoxicity of residual pesticide in onion tips too. It can be reported that residual pesticides extracted from such fruit samples were able to cause genotoxicity in onion root tips and hence can thereby affect other life forms as well. The above results reflect the importance of *Allium cepa* root tips for monitoring genotoxicity (Ping *et al.*, 2012). Use of such plant test systems (*Allium cepa*), is advantageous in terms of reproductive nature, low cost, possibility to be applied *in vivo* and in vitro as well. Moreover, they enable standardization of method in controlled laboratory conditions (Mesi and Kopliku, 2013). With such experiments, preliminary genotoxicity assessment can be easily concluded while avoiding the use of animal model testing. There are also studies stating that cytotoxicity in onion root tips shows similar results to those of *in vivo* testing in animals (Teixeira *et al*., 2003). From the above data (Table 2 and Figure 1), it is evident that genotoxicity studies can be observed using *Allium cepa* root tips under foldscope. Such studies require very basic amenities and avoid handling of toxic compounds which makes it an effective way of detecting and observing genotoxicity of food contaminants (pesticides and preservatives) not only in high-tech research laboratories but in college level practicals as well. Hence, we can conclude that at an optimal concentration of 100 µg/ml, the above experiment can be easily conducted in college level for studying genotoxicity of preservatives and pesticides.

CONCLUSION

Pesticides are potentially toxic to humans and can have both acute and chronic health effects and so are the preservatives. In the above study, among the tested food contaminants, MSG was recorded to be the most toxic. MSG being most widely used in food items, experiments related to its toxicity can be

Table 2. Mitotic index value of different treatments (100µg/ml) as calculated in regular intervals of time

Group	Treatment		Day 1 (24 hours) (%)	Day 2 (48 hours) (%)	Day 3 (72 hours) (%)
1	Control	Distilled water	63±0.8	65±0.6	68±1.1
2	Pesticide	Malathion	23±1.02	18±0.32	16±0.7
		Chlorpyrifos	32±0.54	25±0.7	18±1.12
		Residual pesticide	44±0.85	39±1.01	36±0.69
3	Preservative	MSG	22±0.46	18±0.7	15±1.02
		SB	42±0.32	38±0.56	35±1.32
		PG	36±0.87	31±0.8	29±0.36

* Values in the table are mean ± SD (n=3)

easily conducted due to its easy availability which is also less expensive compared to the purified standards. The above observations related to MSG toxicity in *Allium cepa* bulbs were a result of repeated chronic dose study. Further studies can be conducted targeting toxicity of MSG induced as per daily human consumption. Experiments involving toxicity of relatively lesser toxic food contaminants can be also easily performed by biology students as it would be less hazardous. From the above study, it is also evident that pesticides sprayed on the fruits retain even after thorough washing and can cause toxicity in *Allium cepa* bulbs which is a matter of serious concern. However, the above findings call for more research in this area to further illuminate its extent of effects on the environment.

Thus, we can conclude that the experiment designed for observing genotoxicity of these contaminants in *Allium cepa* model was fruitful and can be further applied for studying toxicity of other contaminants with minimal lab requirements.

ACKNOWLEDGEMENT

The authors are thankful to DBT Foldscope Program, Department of Biotechnology, Govt. of India for the funding and Assam down town University for the infrastructure facilities.

REFERENCES

Abhishek, A., Ansari, N.G., Shankhwar, S.N., Jain, A. and Singh, V. 2014. *In vitro* toxicity evaluation of low doses of pesticides in individual and mixed condition on human keratinocyte cell line. *Biomed. Inform.* 10 (12) : 716-720.

Bolognesi, C., Creus, A., Ostrosky-Wegman, P. and Macros, R. 2011. Micronuclei and pesticide exposure. *Mutagenesis.* 26 (1) : 19-26.

Cabuga, C.C.J., Abelada, J.J.Z., Apostada, R.R.Q., Hernando, B.J.H., Lador, J.E.C., Obenza, O.L.P., Presilda, C.J.R. and Havana, H.C. 2017. *Allium cepa* test: An evaluation of genotoxicity. *Proceedings Int. Aca. Eco. Environ. Sci.* 7 (1): 12-19.

Choudhary, S. and Bhatnagar, N. 2016. Determination of Selected Pesticide Residues in Fruits using Quechers Approach and Reversed-Phase High Performance Liquid Chromatography (RP-HPLC). *Int J. Adv. Sci. Eng. Technol.* 4 (1) : 19-22.

Das, K., Dutta, P. and Gogoi, J. 2019 Foldscope- A simple and economical microscope. *J. Biol. Edu.* doi:10.1080/00219266.2019.1682641.

Grover, I.S., Dhingra, A.K., Adhikari, N. and Ladhar, S.S. 1990. Genotoxicity of pesticides and Plant systems. *Progress Clinical Biol. Res.* 340E : 91-106.

Husarova, V. and Ostatnikova, D. 2013. Monosodium Glutamate Toxic Effects and Their Implications for Human Intake: A Review. *JMED Res.* DOI: 10.5171/2013.6087.

Matsumoto, S.T., Mantovani, M.S., Mirtis Malaguttii, I.A., Dias, A.L., Fonseca, I.C. and Marin-Morales, M.A. 2006. Genotoxicity and mutagenicity of water contaminated with tannery effluents, as evaluated by the micronucleus test and comet assay using the fish *Oreochromis niloticus* and *Chromosome aberrations* in onion root-tips. *Genet. Mol. Biol.* 29 (1): 148-158.

Mahmood, I., Imadi, R.S., Shazadi, K., Gul, A. and Hakeem, R.K. 2015. Effect of pesticides in environment: In: *Plant, Soil and Microbes; Implications in Crop Science*. 1: 253-269.

Mesi (Dizdari), A. and Kopliku, D. 2013 Cytotoxic and Genotoxic Potency Screening of two Pesticides on *Allium cepa* L. *Procedia Technol.* 8 : 19-26.

Pandey, H., Kumar, V. and Roy, B.K. 2014. Assessment of genotoxicity of some common food preservatives using *Allium cepa* L. as a test plant. *Toxicol. Reports.* 1: 300-308.

Patel, D. and Ramani, R. 2017. A Review on *in vitro* Genotoxic Effects of Food Preservatives on Human Lymphocytes. *International Research Journal of Biol. Sci.* 6 (7): 15-18.

Turkoglu, S. 2007. Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. *Genetic Toxicology and Environmental Mutagenesis*. 626 (1-2): 4-14.

Teixeira, R.O., Camparoto, M.L., Mantovani, M.S. and Vicentini, V.E.P. 2003 Assessment of Two Medicinal Plants *Psidium guajava* L. and *Achillea millefolium* L. in *in vitro* and *In vivo* assays. *Gen. Mol. Biol.* 26 : 551-555.

Yuzbasioglu, D., Unal, F. and Sancak, C. 2009. Genotoxic Effects of Herbicide Illoxan (Diclofop-methyl) on *Allium cepa*. L. *Turk. J. Biol.* 33(4) : 283-290.

Viel, A.M., Silva, L.P., Martins, G.R., Urtremari, B., Sekiya, A., Dokkedal, A.L., Sousa, E.D., Camargo, I.S.C. and Silva, R. 2019. Toxicological, Genotoxic and Antioxidant Potential of *Pyrostegia Venusta*. *Bioscience J.* 35 : 570-585.

U.S. EPA. Health and Environmental Effects Profile for Malathion. U.S. Environmental Protection Agency, Washington, D.C., EPA/600/X-84/328 (NTIS PB88180468).

Ping, K.Y., Darah, I., Yusuf, U.K., Yeng, C. and Sasidharan, S. 2012. Genotoxicity of *Euphorbia hirta*: An *Allium cepa* Assay. *Molecules* Doi: 10.3390/molecules17077782.