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Determination of Mitotic Index and Evaluation of Cytogenotoxicity of Oxyfluorfen Using *Allium Cepa* Root Tip Meristematic Cells

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Abstract

The present study aimed to investigate Oxyfluorfen induced cytogenotoxicity employing Allium cepa meristematic cells as test models. The root length inhibition was determined exposing the meristematic cells to eleven Oxyfluorfen concentrations such as 0.01, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9ppm and 1ppm against controls and the inhibition was statistically ($p<0.05$) significant at 0.2, 0.3, 0.4, 0.7, 0.8, 0.9 and 1ppm and insignificant for the rest. The root length was drastically reduced at 0.3ppm and complete at 1ppm. The significant change in cell shape and structure was observed at 0.4 & 0.5ppm when compared to 0.01 & 0.1ppm. The mitotic index was calculated considering interphase, prophase, metaphase, anaphase and telophase as cell cycle stages out of one thousand cells. The per cent mitotic index of 0.01, 0.1, 0.2, 0.3, 0.4ppm and 0.5ppm concentrations was found to be 49, 32.8, 29.4, 0, 32.8 and 51.4 respectively and this result significantly correlates with root length inhibition studies. Cell cycle check points will block or delay the progression of mitosis due to pesticide interference with mitotic phase duration, making some phases more frequent than others. Therefore, it is established that Oxyfluorfen is cytogenotoxic to Allium cepa meristematic cells and this technique is very convenient to assess how toxic substances bring abnormality at cell and organelles level.

Keywords: *Cytogenotoxicity evaluation, Allium cepa, Mitotic index, Root length, Oxyfluorfen*

1. Introduction

Pesticides are intrinsically toxic substances that have been made to protect crops and prevent disease by eliminating various pests and plant diseases. To achieve this target, pesticides normally interrupt certain life processes of pests so that the crop productivity increased (1). Their uses are not only limited to the agriculture but also in houses and workplaces to control mosquitoes, mice and other insects. For this reason, the investigation on non-target organisms is among the popular topics of today's modern life (2). Once they enter in our biological process, it's really difficult to eliminate them from the environment and disturb various biochemical processes leading to fatal results. Thousands of toxic chemicals including pharmaceuticals, pesticides and petroleum products are present in the environment and new chemicals are being introduced every year. In Humans, pesticide exposure may lead to an array of health outcomes, including cancer, respiratory, neurodegenerative, cardiovascular, reproductive, and genetic disorders (3) (4). The type and severity of the hazard may vary depending on the pesticide type, dose, route and duration of exposure (5). Environmental biologists are presently concerned to safeguard the human beings from exposure to chemicals (6) (7).

Previously, different techniques have been developed to evaluate the ecotoxicological effects of pesticides. Among these, *Allium cepa* test is widely employed in xenobiotics and is very sensitive assay and potential biomarker of genotoxicity studies caused by chemicals (8). Further, the species can be used to evaluate DNA damage, such as chromosomal aberrations and disturbances in the mitotic cycle and approved as indicator organisms for bio-monitoring of environmental pollutants (9) (10) (2).

2. Literature Review

The pesticides are numerous kinds and each is active against specific pests (11). The impact of pesticides on crop production is undoubtedly profitable, but they constitute a common and widespread cause of soil, water and air pollution, especially in developing countries. The robust growth in the world economy has led to multifold utilization of agriculture-based chemicals which often induce calamitous effects on the environment (12). Pesticides were deemed inevitable to meet the growing demand for agricultural products because they combat pests that decrease productivity (4). According to FICCI (13), major share in the Indian crop protection market is of insecticides (60%) followed by fungicides (18%) and herbicides (16%) of total pesticides. In 2019, the world consumed about 4.1 million tons of pesticides (14). Besides their use in agriculture, pesticides also control disease vectors (12) and household pests (15).

The most common parameters tested in the *Allium cepa* assay were the damage to cell structure, shape and size, reduction of germination, inhibition of mitotic index, changes in the percentage of mitotic phases, reduction of root growth, induction of nuclear and chromosomal aberrations and DNA damage. Therefore, using *Allium cepa* has been the most efficient since, similar toxic effects are observed in human lymphocytes. The *Allium cepa* species is acknowledged as a bioindicator for evaluating the effect of chemicals, drugs and pesticides to not only for prevention but also to predict the impacts of various substances (16). The different doses of tetraconazole toxicity were evaluated on the *Allium* cepa root tips by means of physiological, cytogenetic, biochemical, and anatomical parameters. It resulted in reduced germination ratio, root length, and weight gain (17) (18). Similarly, the presence of chromosomal aberrations, micronuclei frequency, and mitotic index abnormalities was evaluated on exposure to malathion for 48h to 0.5 mg mL^{-1} and 1.0 mg mL^{-1} (19). The chromosome anomalies to glyphosate exposure reported by Finkler et al (20) are apoptotic bodies, giant cells and nuclear erosions. Pesticides can also directly inhibit tubulin polymerization, which disturbs mitotic spindle formation (21) and consequently inhibits the mitotic spindle (22). Bordin et al (23) reported that the mutagenic effects of the mixture of the herbicides indicating the genetic material toxicity. In view of this, we have tried to understand how Oxyfluorfen is affecting the root length, cell structure and shape, mitotic index, and nucleus of *Allium cepa* meristematic cells in the present studies.

3. Materials And Methods

Oxyfluorfen: Commercial grade Oxyfluorfen (trade name is Galigan) used in this study was manufactured by ADAMA India Private Limited, Bharuch, Gujarat and registered in Hyderabad, Telangana, India. Oxyfluorfen (2-chloro-1-(3-ethoxy-4-nitrophenyl)-4-(trifluoromethyl) benzene), is a diphenyl ether-based herbicide commonly used to control certain annual broadleaf and grassy weeds in vegetables, fruits, cotton, ornamentals and non-crop areas.

Allium cepa: The onion bulbs were selected because of fast growth rate, easiness to handle and convenience of cytological observation. The root tip (meristem) coming out from the common lighted onion (*Allium cepa*) was taken for experiment.

Selection of Rooting Bulbs: Equal size, healthy and red-light bulbs of *Allium cepa* were procured from a local market and devoid of any damage or fungal infection. The onion bulbs were air dried for five days before being stripped of their exterior dry brown scales. To prevent the primordial cells from drying out and to promote the growth of new roots, the dried roots and the outer dead scales of the bulb were carefully removed with a sharp blade and placed in small glass jars of 100ml with basal ends dipping in distilled water and are grown for 7 days at 25±2°C in laboratory (24) (25).

Preparation of suspension for experiment: The Oxyfluorfen stock solution (1000ppm) and other experimental solutions were prepared from stock by dissolving Oxyfluorfen in double distilled water (7). Within 2-3days, the roots grew up to 2-3cm length and were ready to treat with eleven different concentrations such as 0.01ppm, 0.1ppm, 0.2ppm, 0.3ppm, 0.4ppm, 0.5ppm, 0.6ppm, 0.7ppm, 0.8ppm,

0.9ppm, 1ppm and the glass jars were labelled accordingly. The germinated bulbs were removed at the end of seventh day and root length was measured both in control and treated groups with a calibrated ruler. The test solutions were changed every seven days and experiments were repeated four times. The roots were protected from direct sunlight in order to minimize fluctuations in the rate of cell division and the results were reported statistically as Mean ± SD.

Cytogenotoxicity Studies

Excised root tips were fixed for 24h in Clarke's solution (70% ethanol and 30% glacial acetic acid, 3:1). Root tips were first hydrolysed in 2ml of 1N HCl for 5min and incubated in water bath for 12min at 60°C. The HCl is discarded and rinsed with distilled water. Then 2-4 drops of acetocarmine were added and stained for 5-6 minutes. Root tips were then transferred on to glass slides and the tip of root that is stained clearly is cut with a blade. Root tips are covered with a cover slip and squashed by tapping with the end of the histological needle several times to spread the cells evenly and examined under an Olympus binocular stereo microscope (400X). Four bulbs were tested for each concentration and five roots were examined separately for each bulb as suggested by Maria Sabeen et al (26). Cytotoxicity was assessed using the mitotic index (MI), in which the number of cells in cell division stage (prophase, metaphase, anaphase and telophase) in one thousand counted cells was recorded per each treatment. The genotoxic effects were evaluated by recording nuclear changes such as cytoplasmic vacuoles, elongation, condensation and binucleate cells. Mitotic index (MI %) is calculated using following formula as recommended by Finkler et al (20).

$$\text{Mitotic index (MI \%)} = \frac{\text{Number of cells in cell division}}{\text{Total number of counted cells}} \times 100$$

$$\text{Nuclear abnormalities} = \frac{\text{Number of nuclear abnormalities}}{\text{Total number of counted cells}} \times 100$$

3. Results and Discussion

The indiscriminate use of pesticides has often caused poisoning of agricultural crops and environmental contamination. Therefore, the evaluation of Oxyfluorfen toxicity, its action mechanism and consequences on root length, mitotic index, cell shape, structure, growth, and nuclear abnormalities, as performed in the present study, is important in providing useful information for *Allium cepa* cultivation. The average root length of 6-8 roots of each bulb was taken into consideration and the experiment was repeated four times. The observed mean root length to different concentrations of Oxyfluorfen against control is presented in Table-1 and the root length is inhibited in all treated groups concentration dependently but drastically reduced at 0.3ppm and complete at 1ppm. The t- test performed using the above data showed that there is a statistically significant difference ($p<0.05$) for 0.2, 0.3, 0.4, 0.7, 0.8, 0.9 and 1ppm and insignificant for rest of the concentrations. The cell shape and structure got changed more at higher concentrations (0.4ppm & 0.5ppm) compared to that of lower concentrations (0.01ppm & 0.1ppm). So, it can be inferred that the higher concentrations of oxyfluorfen had a greater damage to the cell shape and structure (Table 2).

Table 1: Determination of *Allium cepa* root length on exposure to different concentrations of Oxyfluorfen for 7 days

Days of observation	control	0.01 ppm	0.1 ppm	0.2 ppm	0.3 ppm	0.4 ppm	0.5 ppm	0.6 ppm	0.7 ppm	0.8 ppm	0.9 ppm	1ppm
Week 1	6.1	0	7.1	3.2	0.7	5.8	5.5	6.2	4.5	2.5	5	0
Week 2	3.8	1.8	0.9	2.7	0	2	5.8	4.3	2.4	1.8	1.2	0
Week 3	7.3	4.3	0	4.2	0	5.6	6.3	1.5	1.8	1.8	1.4	0
Week 4	7.2	7.5	0	6.1	0	4.8	1.4	1.3	6	1.4	1.2	0
Mean± SD	6.1± 1.4	3.4± 1.52	2±2 .96	4.05± 1.30	0.17± 0.32	4.55± 1.51	4.75± 1.95	3.32± 2.04	3.67± 1.67	1.87± 0.39	2.20± 1.61	0

Table 2: Morphological manifestations recorded in cell shape and structure due to Oxyfluorfen exposure for 7 days

Types of manifestation	0.01ppm	0.1ppm	0.2ppm	0.3ppm	0.4ppm	0.5ppm
Elongation	31	39	41	0	163	159
Vacuolization	9	10	16	0	41	98
Cell lysis	1	4	1	0	3	14
% abnormal cells	**4.1**	**5.3**	**5.8**	**0**	**20.7**	**27.1**

Based on root length inhibition results, selected Oxyfluorfen concentrations were used to study mitotic index, cell shape, structure and nuclear abnormalities. During mitotic index study, for each concentration, interphase, prophase, metaphase, anaphase, telophase was recorded out of 1000 cells and mitotic index is calculated. The per cent mitotic index of 0.01, 0.1, 0.2, 0.3, 0.4ppm and 0.5ppm concentrations were found to be 49, 32.8, 29.4, 0, 32.8 and 51.4 respectively (Table 3). The per cent MI was least at 0.2ppm and highest at 0.5ppm concentration and this result significantly correlates with our root length studies. Cell division has several cell cycle checkpoints that can block or delay the progression of mitosis due to the presence of cellular and environmental stressors. Thus, pesticides could interfere with mitotic phase duration, making some phases more frequent than others. For example, the increase in prophase cells may result from the CHFR checkpoint blockage that prevents and/or delays the cell from entering the metaphase. Hence, metaphase, anaphase, and telophase indices will be reduced; while there will be an accumulation of cells in prophase (20). The mitodepressive action of pesticides could cause the inhibition of root growth (27) (16), germination, weight gain, root number and finally tissue death (28). The higher mitotic index may be a consequence of a reduction in the time required for DNA repair (29) (7). Similarly, Oxyfluorfen targets a specific enzyme, oxidase, in the chlorophyll biosynthetic pathway. Inhibiting protoporphyrinogen oxidase in plants leads to an accumulation of phototoxic chlorophyll precursors which in the presence of light, produce activated oxygen species which rapidly disrupt cell membrane integrity. Oxyfluorfen must contact plant foliage to cause effects. Plants that are actively growing are most susceptible to Oxyfluorfen (30).

Table 3: Determination of Mitotic Index (% MI) of *Allium cepa* root cells when exposed to different concentrations of Oxyfluorfen for 7 days

Stages of Mitosis	0.01ppm	0.1ppm	0.2ppm	0.3ppm	0.4ppm	0.5ppm
Inter phase	176	132	88	0	132	118
Prophase	164	76	92	0	76	126
Metaphase	110	90	100	0	90	182
Anaphase	34	20	14	0	20	60
Telophase	6	10	0	0	10	28
Mitotic Index (%)	**49**	**32.8**	**29.4**	**0**	**32.8**	**51.4**

The nuclear abnormalities like cytoplasmic vacuoles, elongated nucleus, condensed nucleus, and binucleate nucleus were enumerated for 1000 exposed cells at each concentration and found that the binucleate cells are more at 0.4 and 0.5ppm concentrations and are absent at lower concentrations. The condensed and elongated nucleus was found to be decreased as the concentration increases. The calculated Mean±SD of abnormal nucleus at the concentrations of 0.01, 0.1, 0.2, 0.3, 0.4ppm and 0.5ppm were found to be 26.5±22.06, 16±12.24, 8.5±4.97, 0, 22.5±8.29, 27±17.23 respectively. The total per cent of nuclear abnormalities at 0.01, 0.1, 0.2, 0.3, 0.4 and 0.5ppm were found to be 10.6%, 6.4%, 3.4%, 0%, 9% and 10.8% respectively (Table 4). Several studies have shown that changes in chromosome structure are not just point mutations but also genomic in nature leading to morphological manifestations in chromosomes (31) (32) (33) (34) (35) (23). The chromosome anomalies reported by Finkler et al (20) are apoptotic bodies, giant cells, and nuclear erosions when exposed to different concentrations of glyphosate. Certain chemical compounds also cause microtubule depolymerization effecting mitotic spindle (7). Similarly, tetraconazole induced cytotoxic and genotoxic effects against meristematic cells of *Allium cepa* root tips were reported by Macar (17) by analysing parameters like activities of superoxide dismutase (SOD) and catalase (CAT) enzymes, malondialdehyde (MDA)

content, mitotic index (MI), scores for micronucleus (MN) and chromosomal aberrations (CAs) and results were very significantly impressive.

Table 4: Calculation of nuclear abnormalities in root cells of *Allium cepa* when exposed to Oxyfluorfen for 7 days

Nuclear abnormalities	0.01ppm	0.1ppm	0.2ppm	0.3ppm	0.4ppm	0.5ppm
Cytoplasmic vacuoles	30	18	12	0	18	52
Elongation	60	34	12	0	34	34
Condensation	16	12	10	0	12	10
Binucleate cells	0	0	0	0	26	12
Mean±SD	26.5±22.06	16±12.24	8.5±4.97	0	22.5±8.29	27±17.23
% abnormal cells	**10.6**	**6.4**	**3.4**	**0**	**9**	**10.8**

4. Conclusion

As hypothesized, Oxyfluorfen significantly caused cytogenotoxicity to *Allium cepa* root meristem cells. The root length, size, structure, mitotic index and nuclear changes were observed in concentration and time dependently. During study, it is re-established that there is a positive correlation between the length of root and mitotic index. Hence, mitotic index is instrumental in assessing toxicity in plant growth and developmental studies. Interestingly at 0.3ppm of Oxyfluorfen, the complete inhibition of root growth was noticed in all four replicates and the reasons are to be established. Therefore, it is concluded that oxyfluorfen is cytogenotoxic to *Allium cepa* and care to be taken while applying in agricultural fields.

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References:

1. Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., L. Hens, 2016. Chemical pesticides and human health: the urgent need for a new concept in agriculture. Front. Public. Health., 4:148. https://doi.org/10.3389/fpubh.2016.00148
2. Ciğerci, İ. H., Liman, R., İstifli, E. S., Akyıl, D., Özkara, A., Bonciu, E., & F. Colă, 2023. Cyto-Genotoxic and Behavioral Effects of Flubendiamide in *Allium cepa* Root Cells, *Drosophila melanogaster* and Molecular Docking Studies. Int. J. Mol. Sci., 24(2): 1565
3. Perumalla, V.R., Rahman, M., Mahboob, M., Indu Kumari, S., Chinde, S., & N. Dumala, 2016. Assessment of genotoxicity in female agricultural workers exposed to pesticides. Biomarkers., 22(5): 446–454.
4. Dhananjayan, V. 2020. Agrochemicals impact on ecosystem and bio-monitoring, Resources use efficiency in agriculture. 349-388. Doi:10.1007/978-981-15-6953-1_11
5. Shokunbi, O. 2022. In vivo toxicological assessment of water samples from three farm settlements in *Allium cepa* and *drosophila melanogaster*.
6. Khanna, N., & Sharma, S. 2013. *Allium cepa* root chromosomal aberration assay: a review. Ind. J. pharma. Boil. Res. 1(03): 105-119.
7. Camilo-Cotrim, C. F., Bailão, E. F. L. C., Ondei, L. S., Carneiro, F. M., & L.M. Almeida, 2022. What can the *Allium cepa* test say about pesticide safety? A review. Environ. Sci. Poll. Res. 29(32): 48088-48104.
8. 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. & H.C. Havana. 2017. *Allium cepa* test: An evaluation of genotoxicity. Proc. Int. Aca. Eco. Environ. Sci. 7(1): 12- 19.
9. Kuchy, A.H., Wani, A.A. & A. N. Kamili, 2016. Cytogenetic effects of three commercially formulated pesticides on somatic and germ cells of *Allium cepa*. Environ. Sci. Poll. Res. 23: 6895–6906. https://doi.org/10.1007/s11356-015-5912-6.
10. Gogoi, J. Y., Karabi, D., & P.A. Dutta. 2021. Effect of preservatives and pesticides on mitotic index of *Allium cepa* roots-biological model experiment for genotoxicity. Poll. Res. 40(3): 777-781.
11. Mossa, A. T. H., Mohafrash, S. M., & N. Chandrasekaran. 2018. Safety of natural insecticides: toxic effects on experimental animals. Bio. Med. Res. Int.
12. Konstantinou I, Hela D, Lambropoulou D, & T. Albanis. 2019. Monitoring and assessment of pesticides and transformation products in the environment. Analysis of pesticides in food and environmental samples.2nd edn. CRC Press, Boca Raton. 365-413.
13. FICCI. 2015. Ushering in the 2nd green revolution: role of crop protection chemicals. A report on Indian Agrochemical Industry. 22.

14. FAOSTAT. 2021. Pesticides use: Food and Agriculture Organization. UNO.
15. Mahmood I, Imadi SR, & K. Shazadi. 2016. Effects of pesticides on environment. In: Plant, soil and microbes. Springer Cham, Cham. 253–269.
16. Bosio S, & Laughinghouse IV HD. 2012. Bioindicator of genotoxicity: the *Allium cepa* test. J. Environ. Cont. 137–158.
17. Macar T K. 2021b. Investigation of cytotoxicity and genotoxicity of abamectin pesticide in *Allium cepa* L. Environ. Sci. Poll. Res. 28: 2391–2399. https://doi.org/10.1007/s11356-020-10708-0
18. Çıldır, D. S., & R. Liman. 2020. Cytogenetic and genotoxic assessment in *Allium cepa* exposed to imazalil fungicide. Environ. Sci. Poll. Res. 27: 20335-20343.
19. Ghisi, N. C., Silva, V. B., Roque, A. A., & E. C. Oliveira. 2021. Integrative analysis in toxicological assessment of the insecticide Malathion in *Allium cepa* L. Brazi. J. Biol. 83: e240118.
20. Finkler, M, Gabriela Zimmermann Prado Rodrigues, Juliana Machado Kayser, Ana Luiza Ziulkoski & Günther Gehlen. 2022. Cytotoxic and genotoxic effects induced by associated commercial glyphosate and 2,4-D formulations using the *Allium cepa* bioassay. J. Envron. Sci. Health. Part B. 57(2): 133-141. DOI: 10.1080/03601234.2022.2034432.
21. Verma S, & A. Srivastava. 2018. Morphotoxicity and cytogenotoxicity of pendimethalin in the test plant *Allium cepa* L. A biomarker-based study. Chemosphere. 206: 248-254. https://doi.org/10.1016/j.chemosphere.2018.04.177.
22. Mercado S. A. S., Caleño J.D.Q. & J.P.R. Suárez. 2020. Cytogenotoxic effect of propanil using the *Lens culinaris.* Med and *Allium cepa.* L test. Chemosphere. 249:126193. https://doi.org/10.1016/j.chemosphere.2020.126193
23. Bordin, E. R., Dos Santos, F., Mannes, Y., de Freitas, A. M., & W. A. Ramsdorf. 2023. Single and mixture cytogenetic effects evaluation of atrazine and glyphosate herbicides at environmentally relevant concentrations on *Allium cepa* root meristem cells. Ecotox. Environ. Conta. 18(1).
24. Feretti, D., I. Zerbini, C. Zani, E. Ceretti, M. Moretti & S. Monarca. 2007. *Allium cepa* chromosome aberration and micronucleus tests applied to study genotoxicity of extracts from pesticide-treated vegetables and grapes. Food Additives & Contaminants. 24(6): 561-572, DOI: 10.1080/02652030601113602
25. Alaguprathana, M., M. Poonkothai, Mysoon M. Al-Ansari, Latifah Al-Humaid & W. Kim. 2022. Cytogenotoxicity assessment in *Allium cepa* roots exposed to methyl orange treated with *Oedogonium subplagiostomum*. Environ. Res. 213: 113612. https://doi.org/10.1016/j.envres.2022.113612.
26. Sabeen, M., Qaisar Mahmood, Zulfiqar Ahmad Bhatti, Faridullah, Muhammad Irshad, Muhammad Bilal, Malik Tahir Hayat, Usman Irshad, Tahir Ali Akbar, Muhammad Arslan, & Naeem Shahid. 2020. *Allium cepa* assay based comparative study of selected vegetables and the chromosomal aberrations due to heavy metal accumulation. Saudi J. Biol. Sci. 27(5):1368-1374. https://doi.org/10.1016/j.sjbs.2019.12.011
27. Fioresi, V. S., De Vieira B C R, De Campos J M S & T. S. Da Souza. 2020. Cytogenotoxic activity of the pesticides imidacloprid and iprodione on *Allium cepa* root meristem. Environ. Sci. Poll. Res. 27:28066–28076. https://doi.org/10.1007/s11356-020-09201-5
28. Srivastava, A. K, & D. Singh. 2020. Assessment of malathion toxicity on cytophysiological activity, DNA damage and antioxidant enzymes in root of *Allium cepa* model. Sci. Rep. 10:886. https://doi.org/10.1038/s41598-020-57840-y.
29. Datta, S., Singh, J., Singh, J., Singh, S., & S. Singh. 2018. Assessment of genotoxic effects of pesticide and vermicompost treated soil with *Allium cepa* test. Sust. Environ. Res. 28(4): 171-178.
30. 30. Türkoğlu, Ş. 2012. Determination of genotoxic effects of chlorfenvinphos and fenbuconazole in *Allium cepa* root cells by mitotic activity, chromosome aberration, DNA content, and comet assay. Pest. Biochem. Physiol. 103:224–230. https://doi.org/10.1016/j.pestbp.2012.06.001
31. Freitas, A. S., Cunha, I. M. F., Andrade-Vieira, L. F., & V. H. Techio. 2016. Effect of SPL (Spent Pot Liner) and its main components on root growth, mitotic activity and phosphorylation of Histone H3 in *Lactuca sativa* L. Ecotox. Environ. Saf. 124: 426-434.
32. Palmieri, M. J., Andrade-Vieira, L. F., Trento, M. V. C., de Faria Eleutério, M. W., Luber, J., Davide, L. C., & S. Marcussi. 2016. Cytogenotoxic effects of spent pot liner (SPL) and its main components on human leukocytes and meristematic cells of *Allium cepa*. Water. Air. Soil Poll. 227: 1-10.
33. Bonciu, E., Firbas, P., Fontanetti, C. S., Wusheng, J., Karaismailoğlu, M. C., Liu, D, & A. Papini. 2018. An evaluation for the standardization of the *Allium cepa* test as cytotoxicity and genotoxicity assay. Caryologia. 71(3): 191-209.
34. Liman R, Ciğerci İH & D. Akyıl. 2011. Determination of genotoxicity of Fenaminosulf by *Allium cepa* and Comet tests. Pest. Biochem. Physiol. 99:61–64. https://doi.org/10.1016/j.pestbp.2010.10.006
35. Barbara, L. Oliver Carsten Knörzer, & P. Böger. 1999. Differential Gene Expression in Plants Stressed by the Peroxidizing Herbicide Oxyfluorfen. Z. Naturforsch. 54c: 764 -770.