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Impact of treatment with the insecticide Profen Super on roots of *Allium cepa* L.

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Abstract: *Globally, crop plants are treated with pesticide spray to destroy insects which cause great damage to them. Profen Super is an insecticide that is sprayed on cotton plants to save them from Bollworm attack. In this study rooting of Allium cepa bulbs was allowed to take place in the solution of Profen Super. Four dilutions, 1:1, 1:2, 1:4 and 1:8, of the recommended dose of Profen Super were tested. Root length was the parameter used to study the effect of Profen Super on root growth. Reduction of mitotic index of the cells of the root apical meristem and incidence of chromosomal aberrations were the criteria employed to evaluate cytogenotoxicity of Profen Super by means of Allium cepa assay. The results showed that with insecticide treatment not only was mitotic index much lower in comparison to control but several types of chromosomal aberrations were observed in the cells of the treated roots. Chromosomal aberrations included disturbed prophase, sticky chromosomes, c-metaphase, irregular metaphase, irregular anaphase, multipolar anaphase, chromosomal bridge and vagrant chromosomes. The study shows beyond doubt that all the tested dilutions of Profen Super caused root growth retardation and were cytogenotoxic.*

Key Words: *Allium cepa roots, Profen Super, mitotic index, chromosomal aberrations, cytotoxicity, genotoxicity.*

1. INTRODUCTION:

In modern agriculture, pesticides are used the world over to save crop plants from pathogens and pests. The term pesticides encompasses a wide variety of chemicals that are used as insecticides, fungicides, herbicides, rodenticides, molluscicides, and nematicides [1]. Of these, the most commonly used pesticides are insecticides. To destroy the insects which damage the crop plants, insecticides are generally sprayed on the leaves and other above ground parts of plants. Pesticide use is also common for post-harvest preservation of grain without infestation by pests [2,3]. Though beneficial, pesticides are known to persist in the soil and water, and on fruits and vegetables [4-6]. As a consequence, the chemicals which are utilized to increase the yield of plants get released into the environment. These are harmful not only to humans but also other non-target organisms which is a cause for great concern. The main chemical groups of insecticides are organochlorine, organophosphate, carbamate, pyrethroid, and neonicotinoids [7].

Pesticides are known to be hazardous as they result in several health issues including respiratory disorders and damage to the nervous system [8,9]. Moreover, many pesticides are carcinogenic/ mutagenic [5, 10-12](IARC, 1991; Yu, 2005; Bull et al., 2006; Ferreti et al. 2007). Prenatal exposure to pesticides too may be carcinogenic and even lead to birth defects [5](Ferreti et al. 2007). In the present investigation, the *Allium cepa* assay was used to study the effect of Profen Super, an easily available insecticide. Profen Super, a synthetic broad spectrum insecticide which is chemically an organophosphate, is often used to control bollworm complex pest of cotton though it can also be sprayed on fruits, vegetables and garden plants. It consists of 40% Profenofos and 4% Cypermethrin. Profen Super acts by inhibiting the enzyme acetylcholinesterase and feeding on a treated plant or contact with a treated leaf leads to insect paralysis followed by death [13]. In India, farmers are advised to spray the insecticide cypermethrin + profenofos 44 EC on mustard plants to save them from aphids [14]. Although there are several animal models which have been used to evaluate the genotoxicity of pesticides, ethical concerns about the use of animals have necessitated the use of alternatives to mammals in toxicology studies [15]. The *A. cepa* assay was developed by Levan (1938)[16]. It is an inexpensive, rapid and sensitive test and its results are comparable to those obtained in assays based on other eukaryotic systems [17-20]. The toxic effects of chemical compounds such as depression of mitosis and induction of chromosomal aberrations

in the cells of *A. cepa* root apical meristem can be reliably monitored by this assay [21-27]. Chemicals which induce chromosome aberrations in plant assays are likely to do so in other organisms as well [28,29]. The cytogenotoxicity of many pesticides/ insecticides including deltamethrin [30], ethion [31], imidacloprid [32,33], malathion [34], methiocarb [35,36] and pyriproxyfen [37] has been demonstrated by the *A. cepa* assay. The aim of the present investigation was to study the effect of Profen Super on root growth and division of root apical meristem cells of *A. cepa*. The parameters employed were root length, mitotic index (MI) and chromosomal aberration frequency (CA) as indicators of growth retardation, cytotoxicity and genotoxicity, respectively.

2. MATERIALS AND METHODS:

2.1 Preparation of solutions of Profen Super:

A 35mL/16L water stock solution of Profen Super E.C. (Emulsifiable Concentrate) was prepared with distilled water in accordance with product specification by manufacturer [38]. 1:1, 1:2, 1:4 and 1:8 dilutions of stock solution were prepared with distilled water volume/ volume, respectively.

2.2 Experimental setup:

Medium sized healthy bulbs of *Allium cepa* L. were obtained from the local vegetable market and their older roots and basal tissue plate were removed with a blade to expose root primordia. To set up three replicates of the experiment, three sets of 100 mL beakers were filled with different dilutions of the insecticide solution. *A. cepa* bulbs were allowed to undergo rooting in the insecticide solution by placing them with their bases in contact with the solution. Trays containing the beakers were kept in a plant growth chamber maintained at 26°C, 60% RH and alternating periods of 16h light and 8h darkness. The solution was topped up in the beakers daily to make up for evaporation. For experimental control, the bulbs were placed on beakers containing distilled water. Roots were randomly selected and excised from each bulb on the sixth day, and the lengths of 10 roots from each bulb were measured. The roots were then fixed for 24h in acetic alcohol (45% acetic acid: ethanol, 1:3) fixative. The fixed roots were transferred into screw cap vials containing 70% ethanol and stored in the refrigerator for further study.

1.3 Cytogenetic studies:

Root tips were treated with a solution containing 1N HCl: 45% acetic acid (3:1) to soften the tissue. Temporary squash preparations of root tips stained either with 2% acetocarmine or 2% acetoorcein were then prepared on microslides. The slides were observed under 400X magnification of the microscope. Ten optical fields were randomly chosen for each treatment to score MI and CA. The equations used to calculate MI and CA (Verdes-Teodor *et al*. 2019) are given below:

$$\text{MI} = \frac{\text{number of dividing cells}}{\text{total number of cells}}$$

$$\text{CA} = \frac{\text{number of cells with chromosomal aberrations}}{\text{number of dividing cells}}$$

1.4. Statistical Analysis:

To compare the means for MI and CA values, One-way analysis of variance (ANOVA) and Tukey's post hoc multiple comparison tests were performed at significance level $p < 0.05$. IBM SPSS Statistics-21 software was used for statistical analysis and MS Excel software for plotting the graphs.

3. RESULTS:

Length of root, mitotic index and chromosomal aberration frequency in *A. cepa* were the bioindicators for studying root growth inhibition, cytotoxicity and genotoxicity, respectively, triggered by Profen Super in this investigation. As a consequence of treatment with the insecticide, a decrease in root length was observed with increase in concentration of the insecticide (Table 1, Fig. 1).

Table 1. Effect of different dilutions of Profen Super on average root length, and mitotic index and chromosomal frequency of root meristem cells of *A. cepa*.

Dilution of Profen Super	Average root length (cm)	Mean MI ± SE	Mean CA ± SE
0 (Control)	8.210	0.0434 ± 0.00589	0.00000
1:8	3.645	0.0228 ± 0.00399[a]	0.500 ± 0.06237[b]

1:4	2.125	0.0194 ± 0.00490[a]	0.406 ± 0.06772 [b]
1:2	1.500	0.0206 ± 0.00508[a]	0.590 ± 0.04583[b]
1:1	1.605	0.0196 ± 0.00304[a]	0.898 ± 0.04271[b]

[a, b] Values are significantly different from control at $p < 0.05$

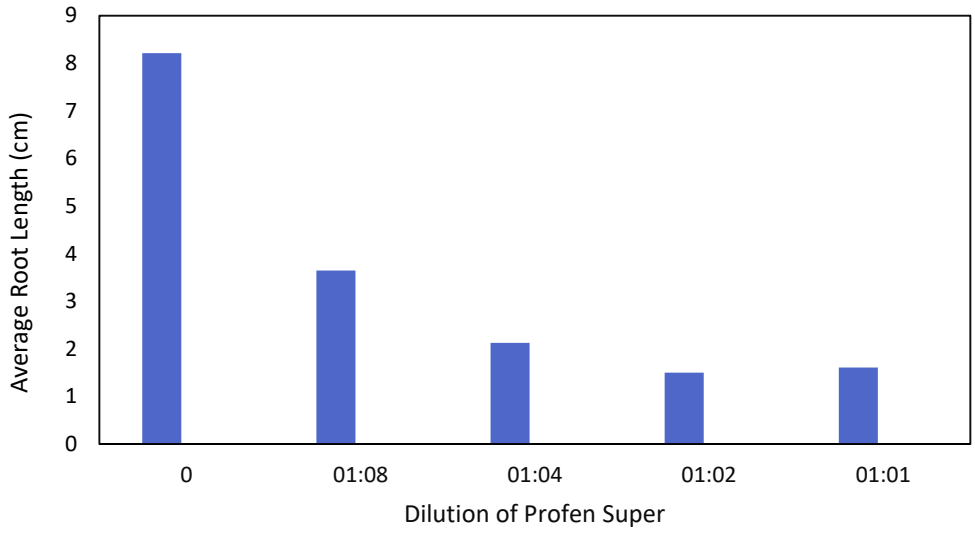

Figure 1 Retardation of root length of *A. cepa* caused by different dilutions of Profen Super.

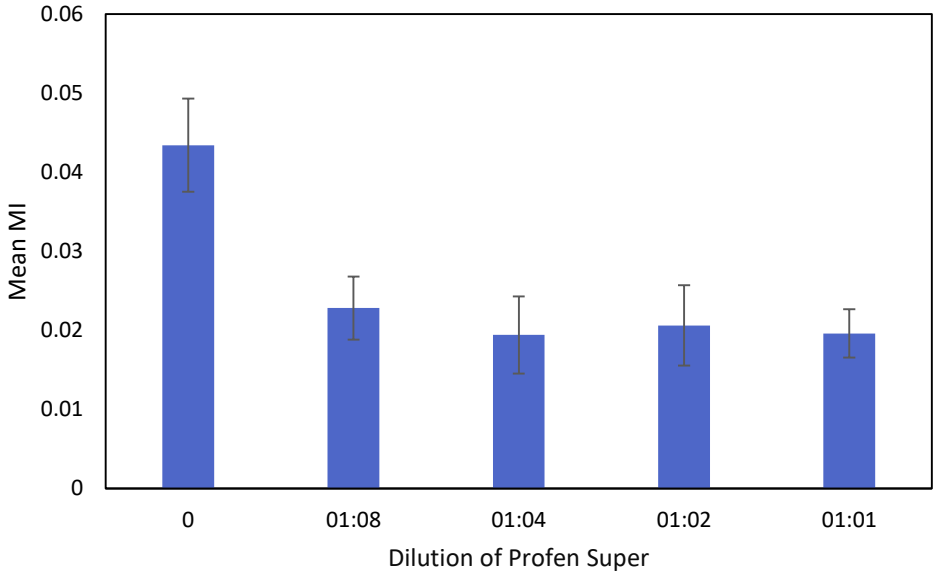

Figure 2. Cytotoxic effect of different dilutions of Profex Super on MI of root meristem cells of *A. cepa*. Standard error of the means is represented by error bars.

Table 1 shows the mean MI ± Standard Error (SE) recorded for root tip cells of *A. cepa*. The mean MI value for untreated control was 0.043. In comparison to control, the mean MI values for 1:8, 1:4, 1:2 and 1:1 dilutions of Profen Super were 0.0228, 0.0194, 0.0206 and 0.0196, respectively (Table 1). Thus, the mean MI for all the dilutions of Profen Super was found to be much lower than control. The difference between mean MI values for control and all dilutions of the insecticide was significant at $P< 0.05$ (Fig. 2). However, the difference between means was not significant for different dilutions of Profen Super. Completely normal mitotic division stages were observed in the meristematic cells of untreated roots (Fig. 3). But treatment with Profex Super resulted in several types of chromosomal aberrations in the meristematic cells which indicated its genotoxicity. The mean CA values for 1:8, 1:4, 1:2 and 1:1 dilutions of Profen

Super were 0.500, 0.406, 0.590 and 0.898, respectively, while the value for control was zero. All values were significant at P< 0.05 (Fig. 4).

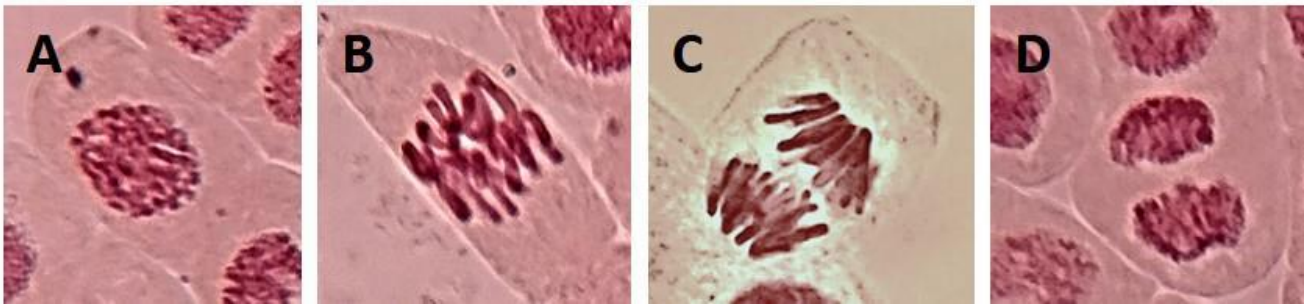

Figure 3. Photographs of normal mitotic stages in the meristematic cells of control roots of *A. cepa.* A. Prophase, B. Metaphase, C. Anaphase and D. Telophase (images at 400X).

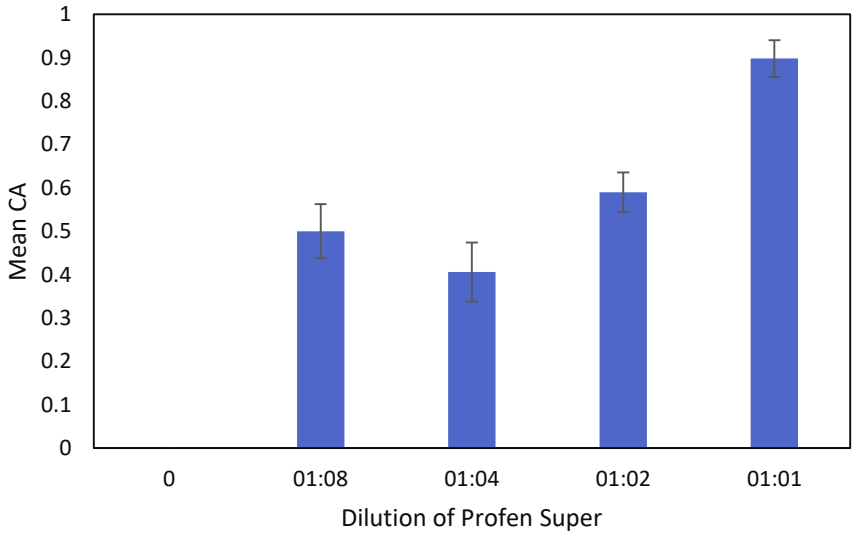

Figure 4. Genotoxic effect of different dilutions of Profen Super on CA of root meristem cells of *A. cepa*. Standard error of the means is denoted by error bars.

An outline of the various chromosomal aberrations seen in response to treatment with different dilutions of Profen Super is given in Table 2. These included disturbed prophase, irregular metaphase, c-metaphase, stickiness of chromosomes, clumping of chromosomes, multipolar anaphase, irregular anaphase, chromosomal bridge and vagrant chromosomes (Fig. 5). The 1:8 dilution of Profen Super produced disturbed prophase, c-metaphase, and irregular metaphase and anaphase. The chromosomal aberrations common to all treatments were c-metaphase and vagrant chromosomes. Sticky chromosomes and chromosomal bridge were observed after treatment with all dilutions except 1:1. In addition to these abnormalities, 1:4 dilution also resulted in multipolar anaphase. Another noteworthy observation was that majority of the cells showed c-metaphase after treatment with 1:4 dilution of the insecticide.

Table 2. Summary of types of chromosomal aberrations recorded in root meristem cells of *A. cepa* following treatment with different dilutions of Profen Super.

S. No.	Dilution of Profen Super	Chromosomal aberrations
1.	0 (Control)	None
2.	1:8	Sticky chromosomes, c- metaphase, irregular metaphase, irregular anaphase, vagrant chromosomes, chromosomal bridge

3.	1:4	Sticky chromosomes, c- metaphase, vagrant chromosomes, asymmetrical anaphase, multipolar anaphase, chromosomal bridge. Majority of the cells showed c- metaphase
4.	1:2	Sticky chromosomes, chromosome clumping, c- metaphase, irregular metaphase, irregular anaphase, vagrant chromosomes, chromosomal bridge
5.	1:1	Disturbed prophase, c- metaphase, irregular metaphase, irregular anaphase, vagrant chromosomes

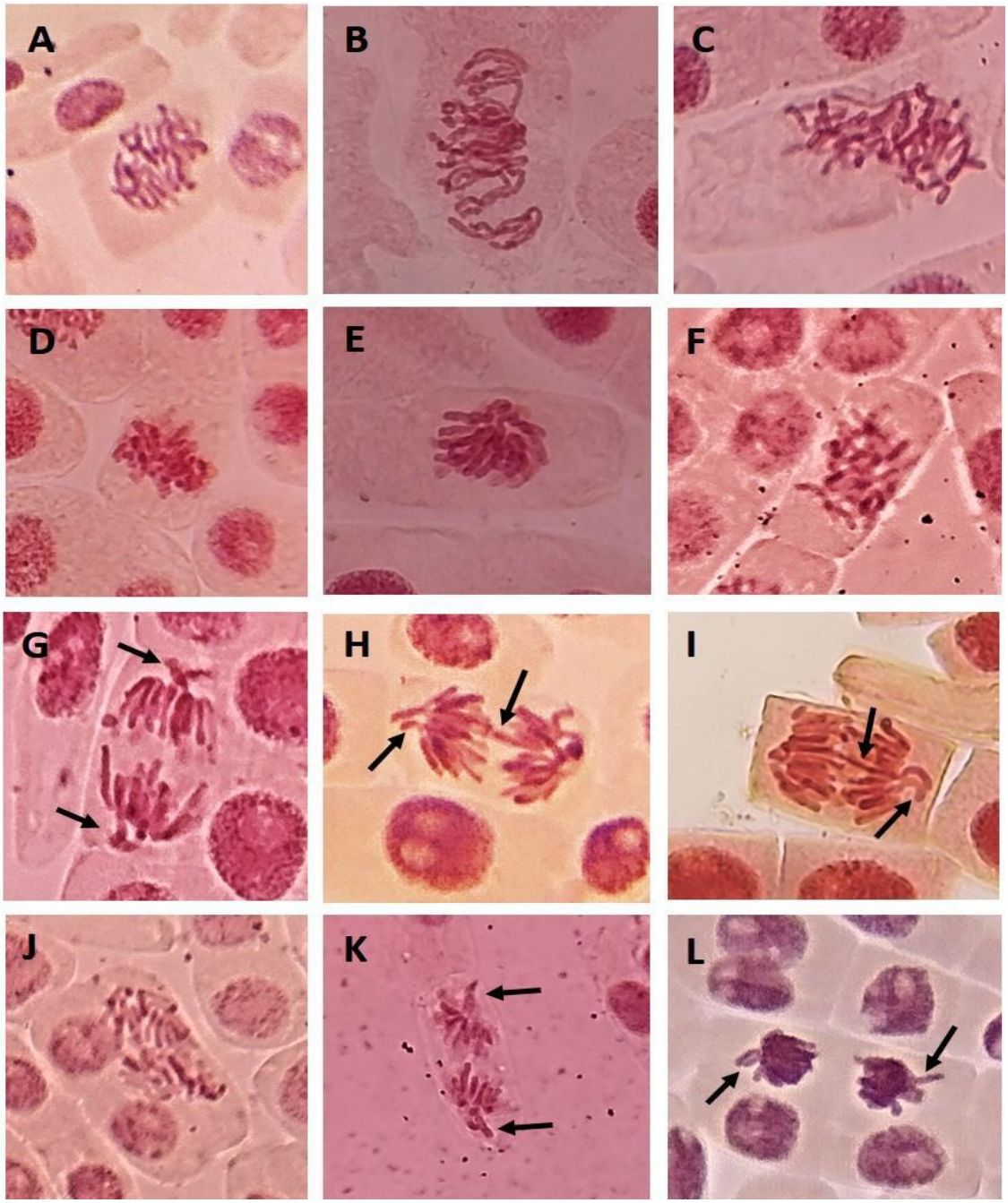

Figure 5 Photographs of meristematic cells of *A. cepa* roots showing different types of chromosomal aberrations induced by treatment with different dilutions of Profex Super. A. disturbed prophase, 1:1; B irregular metaphase, 1:2; C. irregular anaphase, 1:2; D. sticky chromosomes, 1:2; E. clumping of chromosomes, 1:2; F. c-metaphase,1:4; G. vagrant chromosomes, 1:4; H and I. vagrant chromosomes and chromosomal bridge at anaphase, 1:4 and 1:2, respectively; J. multipolar anaphase with vagrant chromosomes, 1:4; K. asymmetrical anaphase with vagrant chromosomes, 1:4; and vagrant chromosomes at late telophase, 1:8 (images at 400X).

4. DISCUSSION:

In the present investigation, root length, MI and CA were used as bioindicators of toxicity of the insecticide Profen Super. One pointer to the toxic nature of this insecticide is the retardation of root growth by all tested dilutions of the insecticide suggesting that it has an inhibitory effect on cells of elongation zone of roots of *A. cepa*. Inhibition of root growth may be attributed to defects in the activity of apical meristem, and delayed cell elongation during differentiation accompanied by loss of cell wall [39]. Reduction of MI and incidence of chromosomal aberrations in meristematic cells have been regarded as measures of cytotoxicity and genotoxicity, respectively, of pesticides in many earlier studies [3,30- 35, 37, 39-46]. Lowering of MI could be the result of disturbed cell cycle due to blockage of cells in G1 or G2 stage, or DNA synthesis repression during S phase [47,48]. Another reason for lowered MI could be arrest of cell cycle at metaphase brought about by c-metaphases which block nuclear division resulting in less development of meristematic tissue [49]. Stickiness of chromosomes, c-metaphase, irregular metaphase and anaphase, multipolar anaphase, chromosomal bridge and vagrant chromosomes are the chromosomal aberrations observed in the root meristematic cells in this study. Stickiness of chromosomes might be due to improper condensation of chromatin fibres causing them to get entangled [50,51]. The chromosomal abnormalities such as c-metaphase, asymmetrical anaphase and multipolar anaphase, indicate disturbance of spindle formation/function [30,41]. Multipolar anaphase could also be the outcome of formation of multipolar spindle which can cause aneuploidy followed by cell death [52]. Disruption of microtubules by the insecticide could lead to spindle fibre dysfunction which would result in irregular anaphase and lack of proper segregation of chromosomes to the poles [53]. Unequal distribution or non-disjunction of chromosomes can cause some chromosomes to move to the pole of the cell ahead of the other chromosomes during anaphase as vagrant chromosomes [54]. Chromosomal bridges observed during anaphase are likely to be the manifestation of chromosome breakage and fusion which lead to multicentric chromosomes being formed [41,55].

5. CONCLUSION:

Profen Super is a broad spectrum insecticide used on crop plants, especially cotton, to save them from insect attack. All dilutions of the insecticide tested in this study led to significant lowering of mitotic index and induced various types of chromosomal aberrations. The results indicate that Profen Super is not only cytogenotoxic to the meristematic cells of *A. cepa* roots but also inhibits the growth of the cells of the elongation zone. This study clearly points to this insecticide being hazardous.

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Conflict of Interest

The authors declare that there is no conflict of interest.

REFERENCES:

1. Bernardes MFF, Pazin M, Pereira LC and Dorta DJ, (2015). Impact of Pesticides on Environmental and Human Health. In Andreazza AC & Scola G. Toxicology Studies-Cells, Drugs and Environment; IntechOpen: London, UK; pp. 195-233. DOI: 10.5772/59710.
2. Raja N, Babu A, Dorn S, lgnacimuthu S, (2001). Potential of Plants for Protecting Stored Pulses from Callosobruchus maculatus (Coleoptera: Bruchidae) Infestation. *Biological Agriculture and Horticulture, 19*, 19-27. http://dx.doi.org/10.1080/01448765.2001.9754906.
3. Asita AO and Mokhobo M M, (2013). Clastogenic and Cytotoxic Effects of Four Pesticides Used to Control Insect Pests of Stored Products on Root Meristems of *Allium cepa*. *Environment and Natural Resources Research, 3*(2), 133-145. DOI:10.5539/enrr.v3n2p133.
4. Tudi M, Ruan HD, Wang L, Lyu J, Sadler R, Connell D, Chu C and Phung DT, (2021). Agriculture Development, Pesticide Application and Its Impact on the Environment. *International Journal of Environmental Research and Public Health, 18,* 1-23. https://doi.org/10.3390/ijerph18031112.
5. Feretti D, Zerbini I, Zani C, Ceretti E, Moretti M and Monarca S, (2007). *Allium cepa* chromosome aberration and micronucleus tests applied to study genotoxicity of extracts from pesticide-treated vegetables and grapes. *Food Additives and Contaminants, 24* (26), 561-572. DOI: 10.1080/02652030601113602.
6. Sharma A, Kumar V, Shahzad B, Tanveer M, Sidhu GPS, Handa N, Kohli SK, Yadav P, Bali AS, Parihar RD, Dar OI, Singh K, Jasrotia S, Bakshi P, Ramakrishnan M, Kumar S, Bhardwaj R and Thukral AK, (2019).

Worldwide pesticide usage and its impacts on ecosystem. *SN Applied Sciences, 1*,1446. https://doi.org/10.1007/s42452-019-1485-1.

7. Mota TFM, Sampaio AR, Vasconcelos MW, de Castilhos and Ghisi N, (2022). *Allium cepa* test vs. insecticides: a scientometric and meta-analytical review. *Environmental Science and Pollution Research, 29*,42678-42691. doi.org/10.1007/s11356-021-15953-5.
8. Kamel F, Engel LS, Gladen BC, Hoppin JA, Alavanja MCR and Sandler DP, (2007). Neurologic symptoms in licensed pesticide applicators in the Agricultural Health Study. *Human & Experimental Toxicology, 26*, 243-250. http://dx.doi.org/10.1177/0960327107070582.
9. Hoppin JA, Umbach DM, London SJ, Henneberger PK, Kullman GJ, Alavanja MCR and Sandler DP, (2008). Pesticides and Atopic and Nonatopic Asthma among Farm Women in the Agricultural Health Study. *American Journal of Respiratory and Critical Care Medicine, 177*, 11-18. http://dx.doi.org/10.1164/rccm.200706-821OC.
10. International Agency for Research on Cancer (IARC), (1991). IARC Monographs on the evaluation of carcinogenic risks to humans. occupational exposures in insecticide application and some pesticides *53*, 33-586.
11. Yu M, (2005). Environmental Toxicology 2nd edition CRC Press, pp. 228-268.
12. Bull S, Fletcher K, Boobis A and Batterrshill J, (2006). Evidence for genotoxicity of pesticides in pesticide applicators. *Mutagenesis, 21*(2), 93-103.
13. https://naclind.com/product/Profen-super/
14. Plant protection portal of Junagarh Agricultural University. http://www.jau.in/index.php/2-init-content/research/93-plant-protection
15. Mukhopadhyay I, Chowdhuri DK, Vajpayee M and Dhawam A, (2004). Evaluation of *in vivo* genotoxicity of cypermethrin in *Drosophila molanogaster* using the alkaline comet assay. *Mutagenesis, 19*(2), 85-90.
16. Levan A, (1938). The effect of colchicine on root mitoses in Allium. *Hereditas, 24*, 471-486. doi.org/10.1111/j.1601-5223.1938.tb03221.x.
17. Leme DM and Marin-Morales MA, (2009). *Allium cepa* test in environmental monitoring: A review on its application. *Mutation Research- Reviews in Mutation Research, 682* (1), 71-81. doi.org/10.1016/j.mrrev.2009.06.002.
18. Macar O, (2020). Multiple toxic effects of tetraconazole in *Allium cepa* L. meristematic cells. *Environmental Science and Pollution Research, 28*(8),10092-10099. doi.org/10.1007/s11356-020-11584-4.
19. Öztürk G, Cavuşoğlu K and Yalcın E, (2020). Dose–response analysis of potassium bromate–induced toxicity in *Allium cepa* L. meristematic cells. *Environmental Science and Pollution Research, 27*(34), 43312–43321. doi.org/10.1007/s11356-020-10294-1.
20. Srivastava AK and Singh D, (2020). Assessment of malathion toxicity on cytophysiological activity, DNA damage and antioxidant enzymes in root of *Allium cepa* model. *Scientific Reports, 10*(1),1-10. doi.org/10.1038/s41598-020-57840-y.
21. Grant WF, (1982). Chromosome aberration assays in *Allium*. A report of the US Environmental Protection Agency Gene-Tox Program. *Mutation Research, 99*(3), 273-291. DOI: 10.1016/0165-1110(82)90046-x.
22. Fiskesjö G and Levan A, (1993). Evaluation of the first ten MEIC chemicals in the *Allium* test. *Alternatives to Laboratory Animals, 21*, 139-149. doi.org/10.1177/026119299302100204.
23. Rank J, (2003). The method of Allium anaphase-telophase chromosome aberration assay. *Ekologija (Vilnius), 1*, 38-42.
24. Trushin MV, Ratushnyak AU, Arkharova IA and Ratushnyak AA, (2013). Genetic alterations revealed in *Allium cepa*-test system under the action of some xenobiotics. *World Applied Sciences Journal, 22*(3), 342-344. doi: 10.5829/idosi.wasj.2013.22.03.2977.
25. Timothy O, Idu M, Olorunfemi DI and Ovuakporie-Uvo O, (2014). Cytotoxic and genotoxic properties of leaf extract of *Icacina trichantha* Oliv. *South African Journal of Botany, 91*, 71-74. doi:10.1016/j.sajb.2013.11.008.
26. Palmieri MJ, Andrade-Vieira LF, Trento MVC, Eleutério MWF, Luber J, Davide LC and Marcussi S, (2016). Cytogenotoxic effects of Spent Pot Liner (SPL) and its main components on human leukocytes and meristematic cells of *Allium cepa*. *Water, Air, & Soil Pollution, 227*, 1-10. doi.org/10.1007/s11270-016-2809-z.
27. Macar TK, Macar O, Yalcın E and Cavuşoğlu K, (2019). Resveratrol ameliorates the physiological, biochemical, cytogenetic, and anatomical toxicities induced by copper (II) chloride exposure in *Allium cepa* L. *Environmental Science and Pollution Research, 27*(1), 657–667. https://doi.org/10.1007/s11356-019-06920-2.
28. Grant WF, (1978). Chromosome Aberration in plants as monitoring system. *Environmental Health Perspectives, 27*, 37-43. http://dx.doi.org/10.1289/ehp.782737.

29. Camilo-Cotrim CF, Bailão EFLC, Ondei LS, Carneiro FM and Almeida LM, (2022). What can the *Allium cepa* test say about pesticide safety? A review. *Environmental Science and Pollution Research, 29*, 48088–48104. doi.org/10.1007/s11356-022-20695-z.
30. Chauhan LKS, Dikshith TSS and Sundararaman V, (1986). Effect of deltamethrin on plant cells I. Cytological effects on the root meristems of *Allium cepa. Mutation Research, 171,* 25-30.
31. Lamsal K, Ghimire BK, Sharma P, Ghimiray AK, Kim SW, Yu CY, Chung IM, Lee YS, Kim J-S and Shakya SR, (2010). Genotoxicity evaluation of the insecticide ethion in root of *Allium cepa* L. *African Journal of Biotechnology, 9*(27), 4204-4210.
32. Rodríguez YA, Christofoletti CA, Pedro J, Bueno OC, Malaspina O, Ferreira RAC and Fontanetti CS, (2015). *Allium cepa* and *Tradescantia pallida* bioassays to evaluate effects of the insecticide imidacloprid. *Chemosphere, 120*, 438–442.
33. Fioresi VS, Vieira BCR, de Campos JMS and Souza TS, (2020). Cytogenotoxic activity of the pesticides imidacloprid and iprodione on *Allium cepa* root meristem. *Environmental Science and Pollution Research, 27*(22), 28066-28076. https://doi.org/10.1007/s11356-020-09201-5.
34. Gogoi P, Das S, Das S and Khan MZA, (2016). Effect of Organophosphorus Insecticide, Malathion on the Division of Meristems of *Allium cepa* L. *International Journal of Pure and Applied Bioscience, 4*(4), 114-122. DOI: http://dx.doi.org/10.18782/2320-7051.2342.
35. Tütüncü E, Yalçin E, Acar A, Yapar K and Çavuşoğlu K, (2018). Investigation of the Toxic Effects of a Carbamate Insecticide Methiocarb in *Allium cepa* L. *Cytologia, 84*(2), 113-117. Doi: 10.1508/Cytologia.84.113.
36. Viteri DM and Linares-Ramírez AM, (2022). Timely Application of Four Insecticides to Control Corn Earworm and Fall Armyworm Larvae in Sweet Corn. *Insects, 13,* 278. https://doi.org/10.3390/insects13030278.
37. Karaismailoğlu MC, (2016). The Evaluation of the Genotoxic and Cytotoxic Effects of Pyriproxyfen Insecticide on *Allium cepa* Somatic Chromosomes with Mitotic Activity, Chromosome Abnormality and Micronucleus Frequency. *Turkish Journal of Life Sciences, ½,* 65-69.
38. https://www.indiamart.com/proddetail/profenofos-40-cypermethrin-4-ec-20111666097.ht
39. Ciğerci IH, Liman R, İstifli ES, Akyıl D, Özkara A, Bonciu E. and Cola F, (2023). Cyto-Genotoxic and Behavioral Effects of Flubendiamide in *Allium cepa* Root Cells, Drosophila melanogaster and Molecular Docking Studies. *International Journal of Molecular Sciences, 24*, 1565. https://doi.org/10.3390/ijms24021565.
40. Al-Ahmadi MS, (2013). Cytogenetic Effects of Two Synthetic Pesticides on Mitotic Chromosome on Root Tip Cells of *Allium cepa. Cytologia, 78*(1), *3-8.*
41. Ahmed FAW, (2014). Cytotoxic and Genotoxic Potency Screening of WIDE-SPEC Pesticide on *Allium cepa* L. Root Meristem Cells. *Journal of Natural Sciences Research, 4*(24), 100-108.
42. Pandir D, (2018). Assesment of the Genotoxic Effect of the Diazinon on Root Cells of *Allium cepa* (L.). *Brazilian Archives of Biology and Technology, 61*, e18160390. DOI:10.1590/1678-4324-2018160390.
43. Akyil D, (2021). Mutagenic and cytotoxic activity of insecticide Napoleon 4EC in *Allium cepa* and Ames test. *Caryologia. International Journal of Cytology, Cytosystematics and Cytogenetics, 74*(2), 11-19. DOI: 10.36253/caryologia-1013.
44. Asita AO and Matebesi LP, (2010). Genotoxicity of hormoban and seven other pesticides to onion root tip meristematic cells. *African Journal of Biotechnology, 9* (27), 4225-4232.
45. Topcu S, Yilmaz F, Ergene A and Tan S, (2013). Genotoxic effects of Dursban 4 in *Allium cepa*. *Hacettepe Journal of Biology & Chemistry, 41*(1), 21-27.
46. Das L, Hasnu S, Shandilya ZM and Tanti B, (2017). Cytotoxic Effect of Malathion and Furadan on *Allium Cepa* L. and Growth Parameters of *Oryza Sativa* L. *International Journal of Multidisciplinary Approach and Studies, 4*(4), 61-75.
47. Sudhakar R, Gowda N and Venu G, (2001). Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*. *Cytologia, 66*, 235-239. https://doi.org/10.1508/cytologia.66.235.
48. Gupta K, Mishra K, Srivastava S and Kumar A, (2018). Cytotoxic Assessment of Chromium and Arsenic Using Chromosomal Behavior of Root Meristem in *Allium cepa* L. *Bulletin of Environmental Contamination and Toxicology, 100*, 803-808. doi.org/10.1007/s00128-018-2344-2.
49. de Oliveira C, Ramos SJ, Siqueira JO, Faquin V, de Castro EM, Amaral DC, Techio VH, Coelho LC, e Silva PHP, Schnug E and Guilherne LRG, (2015). Bioaccumulation and effects of lanthanum on growth and mitotic index in soybean plants. *Ecotoxicology and Environmental Safety, 122*,136-144. doi.org/10.1016/j.ecoenv.2015.07.020.
50. McGill M, Pathak S and Hsu TC, (1974). Effects of ethidium bromide on mitosis and chromosomes, A possible material basis for chromosome stickiness, *Chromosoma, 47*, 157-167. DOI: 10.1007/BF00331803.

51. Klasterska I, Natarajan AT and Ramel C, (1976). An interpretation of the origin of subchromatid aberrations and chromosome stickiness as a category of chromatid aberrations, *Hereditas, 83*, 153-162.
52. Vitre B, Taulet N, Guesdon A, Douanier A, Dosdane A, Cisneros M, Maurin J, Hettinger S, Anguille C, Taschner M, Lorentzen E and Delaval B, (2020). IFT proteins interact with HSET to promote supernumerary centrosome clustering in mitosis. *EMBO Reports, 21*(6), 1-15. DOI 10.15252/embr.201949234.
53. Singh D and Roy BK, (2017)**.** Evaluation of malathion-induced cytogenetical effects and oxidative stress in plants using *Allium* test. *Acta Physiologiae Plantarum 39:* 92-102. DOI:10.1007/s11738-017-2391-z.
54. Dutta J, Ahmad A and Singh J, (2018). Study of industrial effluents induced genotoxicity on *Allium cepa* L. Caryologia: *International Journal of Cytology, Cytosystematics and Cytogenetics, 71*(2),139-145. https://doi.org/10.1080/00087114.2018.1447631.
55. Fenech M, Knasmueller S, Bolognesi C, Holland N, Bonassi S and Kirsch-Volders M, (2020). Micronuclei as biomarkers of DNA damage, aneuploidy, inducers of chromosomal hypermutation and as sources of pro-inflammatory DNA in humans. *Mutation Research-Reviews in Mutation Research, 786*, 108342. DOI: 10.1016/j.mrrev.2020.108342.