



Cytogenetic effects of quinalphos 25% EC on the root tip cells of *Allium cepa* L.

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Abstract

Quinalphos 25% EC is an organophosphorus (OP) insecticide used to effectively control plant hoppers, caterpillars, leaf miners, sucking pests and boll worms in various crops like vegetables, plantation crops, fruit crops, paddy and sugarcane. The present investigation is conducted to investigate the cytogenetic effects induced by Quinalphos 25% EC on the root tip cells of *Allium cepa* L. In this study, mitotic indices and cytogenetic abnormalities of root tip cells of *Allium cepa* L. were evaluated with the different concentrations of test solutions of Quinalphos 25% EC and duration of treatment. It was noted that the test solutions remarkably reduced the mitotic indices and increased the abnormalities in a dose dependent and time dependent manner. The results of the study suggests the intensive study of mutagenic potential of Quinalphos 25% EC on the non-target systems like other animals and humans as its regular and vast application is a potential threat to the genetic constitution of them.

Keywords: organophosphorus pesticides, quinalphos 25% EC, *Allium cepa* L., mitotic index, cytogenetic abnormalities

Introduction

The agrochemicals are commonly used to reduce or eliminate organisms which transmit diseases or destroys agricultural crops and stored products. In developing countries like India, increasing population and decline in land has posed an extra burden on the agriculture. Therefore, the land available for agriculture should be economically utilized and maximum results be obtained [9]. In this context, people turned using agrochemicals widely and thus it became a common practice.

Pesticides constitute a heterologous category of agrochemicals specifically designed to control pests. The synthetic pesticides are synthesized chemically and contain carbon in their chemical structure. Upon the chemical composition of the Active Ingredient (AI), synthetic pesticides are categorized into Organochlorines, Organophosphates, Organosulfurs and so on. Quinalphos 25% EC is an organophosphorus or Organophosphate (OP) insecticide with quick knockdown action against pests such as plant hoppers, caterpillars, leaf miners, sucking pests and boll worms that affects crops like vegetables, plantation crops, fruit crops, paddy and sugarcane. The chemical formula is $C_{12}H_{15}N_2O_3PS$ and the IUPAC name is O, O-diethyl O-quinaxalin-2-yl phosphorothioate. In the World Health Organization's (WHO) acute hazard ranking, Quinalphos 25% EC is ranked as 'moderately hazardous' and is either banned or restricted in many countries. In India, Quinalphos is classified and traded as a yellow label (highly toxic) insecticide.

Many Organophosphorus pesticides reported to be environmental pollutants with mutagenicity, are found to induce chromosomal aberrations and gene mutations of varying magnitudes [8]. The International Agency for Research on Cancer (IARC) has reviewed the potential carcinogenicity of several organophosphate insecticides [7]. Recent findings suggest the necessity to identify compounds that react with DNA in order to assure the environmental quality has led to the development of

several genotoxicity and mutagenicity assays in a wide range of organisms [2].

Plant-based bioassays have recently gained remarkable popularity among the eco-toxicological assessment procedures [1]. The *Allium cepa* has been regarded as one of the most favorable test system to assess chromosomal damages and disturbances in mitotic cycle due to the presence of good chromosomal conditions such as large chromosomes and in a reduced number ($2n=16$) [4]. *Allium* test is proven as sensitive in monitoring the genotoxicity of pesticide residues [3]. Among the 148 chemicals evaluated by *Allium* test, 76% presented positive results, which led the author to suggest it as a standardized test to determine chromosome damages induced by chemicals [11].

Materials and Methods

For the present work, healthy bulbs of *Allium cepa*, L. ($2n=16$) commonly called Onion, belonging to family Liliaceae was chosen as test organism. The chemical used as test solution was varying concentrations of Quinalphos 25% EC, Clarke's fixative, Ethyl alcohol 70%, 1N HCl and Acetocarmine 2% were also used. Four different concentrations (0.025%, 0.05%, 0.075% and 0.1%) of Quinalphos 25% EC were used in the treatment of roots tips cells of bulbs of *A. cepa* L. 0.025% was prepared by mixing 25 μ l of Quinalphos 25% EC and rest as distilled water. Similarly 0.05%, 0.075% and 0.1% of test solutions were prepared. For the test, protocols of [6] with slight modification were used. The bulbs were kept on water soaked, absorbent cotton for 2 days. After 2 days, the bulbs with new roots were selected and transferred to Petri dishes containing specific concentrations of test solutions (ranging from 0.025-0.1%). Bulbs were allowed to grow in the test solutions for four different durations (i.e., 4 hrs, 6 hrs, 12 hrs and 24 hrs) at 28-30°C. A set retained in distilled water served as control. At the end of exposure period to insecticide, were washed

and the roots were carefully excised from the bulb. They were fixed in freshly prepared Clarke's fixative and then transferred to 70% ethyl alcohol and stored in a refrigerator. Upon examination, fixed roots were washed, blotted to dry and hydrolyzed in 1N for 5 min at 60°C which is followed by washing and staining roots with Acetocarmine 2%. Each of the stained root tips were transferred to slides where their deeply stained tip portion is cut off and taken for squash preparation by pressing a cover slip over it. The cover slips were sealed on the slides with nail polish^[10] to prevent drying out.

The slides were examined under a compound microscope. At least 1000 cells (about 200 cells scored per slide) for each concentration and control were analyzed at x 400 magnification. Mitotic Index/MI (%) of each treatment was calculated^[4, 5] as,

$$\text{Mitotic Index/MI (\%)} = \frac{\text{Number of dividing cells}}{\text{Total number of cells observed}} \times 100$$

The different types of aberrations were studied in detail and the frequency of aberrations (%) was calculated using the formula,

$$\text{Percentage of aberrations} = \frac{\text{Number of aberrant cells}}{\text{Total number of cells observed}} \times 100$$

Table 1: Effect of Quinalphos 25% EC on the Mitotic indices of root tip cells of *A. cepa* L. at various concentrations of Quinalphos 25 % EC and duration of treatment.

Concentration of test solution (Quinalphos 25 % EC)	Mitotic Indices (%) in each duration (hrs) of treatment			
	4 hrs	6 hrs	12 hrs	24 hrs
0.025 %	53.14 %	48.09 %	43.72 %	31.28 %
0.05%	52.48 %	48.04 %	42.20 %	31.58 %
0.075%	51.26 %	46.44 %	40.95 %	29.50 %
0.1 %	50.24 %	45.32 %	40.28 %	28.58 %
Control	86.60 %	86.41 %	86.59 %	88.05 %

Table 2: Frequencies (%) of cytogenetic abnormalities noted on *A. cepa* root tip cells with respect to concentration of test solution and duration of treatment.

Concentration of test solution (Quinalphos 25 % EC)	Frequency (%) of cytogenetic abnormalities noted in each duration (hrs) of treatment			
	4 hrs	6 hrs	12 hrs	24 hrs
0.025 %	2.77 %	8.32 %	12.17 %	14.05 %
0.05 %	3.26 %	9.69 %	12.37 %	15.76 %
0.075%	5.33 %	10.76 %	13.54 %	15.06 %
0.1 %	7.93 %	11.86 %	14.54 %	16.63 %
Control	0.04 %	0.043 %	0.53 %	0.91 %

The different types of aberrations recorded were nuclear lesions, binucleate or multinucleate cells, cells with prematurely condensed nuclei, nucleoids, nuclear disintegration, bizarre nuclei and giant cells. Sticky prophase, prophase arrest, telomere puffing, Sticky metaphase, C-mitosis, Multipolar anaphase, sticky anaphase, star anaphase, anaphase bridges, tropokinesis and Laggards, Diagonal telophase, Sticky telophase and telophase with delecting cytokinesis.

In conclusion, the results of this study shows the mitodepressive as well as the cytotoxic and genotoxic potential of Quinalphos 25% EC insecticide on *Allium cepa* which could present toxic risks on human health and environment. Therefore, it is suggested to test the mutagenic potential of Quinalphos 25% EC intensively furthermore its judicial application is recommended in the agricultural practices.

Conclusion

The use of organophosphorus pesticides has assumed considerable significance in modern agricultural practices. However the extensive use of these pesticides results in their widespread distribution in the environment and also creates many problems such as toxicity to non-target organisms, persistence and combined effects with other agro-biochemicals and environmental factors. The *A. cepa* assay has been used widely to study genotoxicity of many pesticides revealing that these compounds can induce chromosomal aberrations in root meristems of *A. cepa*,^[3]. This test provides an important method for screening environmental contamination and their results can be used as a warning to other test systems^[2].

The present investigation on effect of Quinalphos 25% EC in the root tip cells of *Allium cepa* showed there is a significant difference in the mitotic indices of control and treated groups (Table 1). The values of mitotic index decreased with increasing concentrations of the test solutions and duration of the exposure. Furthermore the test solutions induced various types of cytogenetic abnormalities on root tip cells of *A. cepa* with frequencies or percentage of abnormalities are given in the Table 2.

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