CHAPTER -6

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6.1 Summary

The application of insecticides has become an integral part of the modern agriculture. Though the use of insecticides has become a necessity, the frequent and indiscriminate use of these chemicals produce many undesirable secondary consequences on plants, animal and human. Even the frequent use in the recommended doses may induce some of anomalies which may cause some problems in maintaining the genetic stability of the agricultural crops. As the new insecticides are being used regularly in agricultural crops, it is pertinent to investigate the cytotoxicity as well as the mutagenecity of various insecticides used regularly in agricultural crops.

Since insecticides are continuously appearing for commercial use in the agricultural fields, the investigation of their cytotoxicity assume great significance owing to their deleterious effects. With this the present investigation was initiated to test the various aspect of cytotoxic effects of three insecticides in plants on the following parameters:

- Biological effects
- Cytological effects
  i. Effects on mitotic chromosome
  ii. Effects on meiotic chromosome
- Effects on total DNA and RNA content
- Effects on total protein content

In the present investigation the experimental plant materials used were seeds of *Allium cepa* L. (Onion) CV-Rasna-53 and *Nigella sativa* L. (Black cumin).

Three insecticide belonging to the organophosphorus group were used. These were Phosphamidon, Monocrotophos and Phosalone.

Six different concentrations of each of these insecticides were used and these
concentration were 0.10%, 0.20%, 0.30%, 0.40%, 0.50% and 0.60%. Each solution was prepared with pH 5.0, pH 7.0, pH 9.0 and with distilled water alone. Thus there were total 72 treatment combinations for *Allium cepa* L. and as well as for *Nigella sativa* L. (3 insecticides X 6 concentration X 4 levels = 72)

Seeds treated with different treatment combinations were allowed to germinate separately on moist filter paper in petriplates. Untreated (control) seeds were also germinated similarly. Emergence of radicle was taken as the indication of germination. Germinated seeds in each treatment combinations were made into three equal parts. One part was kept in petriplates as it was used for measuring the biological effects of the insecticides. The seeds of second part were allowed to grow for ten days and from the eleventh day onwards root tips were collected from these germinated seeds and were used for cytological studies. The third part was sown in the field. In the field the flower buds of these plants were treated with insecticides solution. After the insecticide treatment, the flower buds were collected and fixed with Carnoy’s fluid and cytotoxicity of the insecticides were measured in terms of chromosomal abnormalities during both mitotic and meiotic cell division, alteration of mitotic index, DNA, RNA and protein content, pollen sterility and chiasma frequency. Biological effects were measured from the following parameters:

- Rate of seed germination
- Seedling survivality
- Seedling injury measured in terms of root and shoot length reduction.

Mitotic index was expressed as relative division rate (RDR) and was calculated as follows:

$$\text{RDR} = \frac{\% \text{ of dividing cell in treated variant} - \% \text{ of dividing cell in control variant}}{100 - \% \text{ of dividing cell in control variant}} \times 100$$

Chromosomal abnormalities were studied during both mitotic and meiotic cell division. The squash and smear preparations were made with aceto-orcein (1.5%) and aceto-carmine respectively. Pollen sterility was studied by staining the pollen grains;
with 4% iodine potassium iodate solution; DNA and RNA content was extracted from the leaf of Allium cepa L. and Nigella sativa L. The protein content was extracted from the roots of both Allium cepa L. and Nigella sativa L. The estimation was done colorimetrically.

With regard to the biological effects, there was a slide decline in the rate of germination in both Allium cepa L. and Nigella sativa L. The treated plants also showed retardation of growth expressed in terms of seedling injury. The rate of growth was found to be lower in higher concentrations of all the insecticides. The pH of the insecticides solutions have pronounced effects. The pH 5.0 of the solution was found to be more effective in lowering the growth rate.

The root tip cells of the treated plants showed various types of chromosomal abnormalities. These were grouped into three categories depending upon the mode of action of the insecticides.

The first group includes those abnormalities which were due to the action of the insecticides on the spindle apparatus. This group included C-metaphase (Colchicine type metaphase), C-anaphase (Colchicine type anaphase) multipolarity, laggards, polyploidy, binucleated cells, micronuclei, unoriented chromosomes, and unequal distribution of chromosomes.

The second group consisted of those abnormalities which involved individual chromosome and were: Fragmentation (chromosome breaks), chromosome bridge (link between two groups of chromosome in anaphase or telophase), and ring chromosome.

The third group included sticky chromosome and clumping of chromosomes.

The mitotic index which was expressed as relative division rate (RDR) and the relative abnormality rate (RAR) was found to be effected by the insecticide treatments. The RDR and RAR also influenced by the pH levels of the insecticide solutions.

The pollen mother cells (PMCs) from the untreated (control) plants showed normal chromosome behaviour during the meiotic division in both Allium cepa L. and Nigella sativa L. The PMCs in the insecticide treated plants showed different consequences
which were of three types depending on the mode of action of the insecticides. The first
group resulted from the action on the spindle apparatus and included the following:
laggard multipolarity, cells with variable number of chromosomal groups with varying
chromosome number, non synchronous chromosome division, formation of micronuclei,
unequal disjunction of chromosome, oblique spindle, cells with double chromosome
number.

The second group consisted of the effect of the insecticide treatments on individual
chromosome and included the following types: chromosome bridge, fragmentation,
univalent and multivalent.

The third group of abnormalities were those that effect the whole chromosome
set and they were sticky chromosome clumping and erosion of chromosome.

The chiasma frequency was also lowered by the insecticide treatments in both the
plant Allium cepa L. and Nigella sativa L. in comparison with the control. The pollen
viability was also found to be effected by the insecticide treatments. In the insecticide-
treated plants the pollen viability was lower. The pollen viability was reduced with the
rise of the insecticide concentrations.

The total DNA content of the plants were also found to be effected by the treatments
with different insecticides. Higher concentrations of the insecticides were effective in
lowering the DNA amount.

The total RNA content of the plants were also affected by the insecticidal treatment both in
Allium and Nigella.

The protein content was found to be reduced by the treatments with different
insecticides.

All the three insecticides viz. Phosphamidon, Monocrotophos and Phosalone had
prominent effects on the mitotic and meiotic chromosome and on the total content of
DNA, RNA and protein.

In all the cases it was found that the concentrations of the insecticides and pH
levels of the treated solution had pronounced effects on both the plants. Generally,
abnormality rate was increased along with the increase of the insecticide concentration.
Higher concentrations of the insecticide solution were found to be more prominent in the induction of cytotoxic effects. The pH levels of the insecticide solution had also similar effects on both the plants. The insecticides prepared with distilled water showed minimum effects; whereas all other solutions with pH 5.0, 7.0 and 9.0 had higher effects on the chromosome as well as on the total amount of nucleic acid and protein. The insecticide solution pH 5.0 were injurious in bringing a higher magnitude of cytotoxic effects. This may be attributed to the acidic nature of the insecticide solutions.

6.2 Conclusion:

It was immersed from the present investigation that phosphamidon, Monocrotophos, and Phosalone were equally effective in inducing cytotoxic effects in the economic plants. However, the magnitude of such effects was minimum at lower concentrations. Cytotoxic effects were slightly more with solution having neutral and alkaline pH. Insecticides caused minimum effect when they were prepared with distilled water alone. All the concentrations of the three insecticides used in the investigation induced cytotoxic effects, although the effects varied depending upon the concentrations. All the concentration or doses tested in the study were within the recommended doses for using in the economic plants. Therefore, it is clearly immersed that insecticides even with the recommended doses are capable of producing cytotoxic effects in the plants. Such cytotoxic effects may ultimately lead to the unstability in the genetic architecture of the plants. Therefore, with prolong use of the insecticides there may be genetic degradation of the character in economic plants. The deleterious effects of the insecticides can not be modified by simply adjusting the pH level of the solutions, however, insecticides solution can be prepared with distilled water alone since such solution yielded minimum effects.

The intensive modern agricultural system enhanced the attack of insect pests. It requires the use of huge amount of insecticides. The consequence is not only lowering
the total yielding of crops but also causes loss of huge amount of money and emergence of new resistant races of insect pests. So it is an endless fight with the insect pests. This completed the introduction new generation insecticides. So by the use of insecticides alone this problem can not be solved. For effective control of insects it requires the introduction of resistant variety of crops which can be developed by transferring the resistant genes or by using non conventional tools like mutation breeding techniques, as well as by new technique of biological control.