AN OVERVIEW OF MUTAGENIC POTENTIAL OF PESTICIDES

Aurel Popescu*, Anca Nicoleta Şuțan*, Elena Popa*, Andreea Cristina Vasile*

*University of Pitesti, Faculty of Sciences, Department of Natural Sciences
Târgul din Vale str., no. 1, 110040 Pitesti, Romania
E-mail: aurel_n_popescu@yahoo.com

Abstract
This paper presents a synthesis of mutagenic potential of a few pesticides. Cytotoxicity tests, using plant test systems in vivo, such as Allium cepa, are validated by the similar results performed in animal testing in vitro. Cytogenetic tests are usefulness for identifying and evaluating the damaging effects of pesticides present in various concentrations under different exposure times on living organisms. Mutagenic potential of different pesticides used can be detected cytologically by cellular inhibition (mitotic index and replication index are used as indicators of adequate cell proliferation), disruption in metaphase, induction of chromosomal aberrations, numerical and structural, ranging from chromosomal fragmentation to the disorganization of the mitotic spindle, and consequently of all subsequent dependent mitotic phases.

Keywords: Allium cepa test, pesticides, cytogenetic effects, mitotic index, chromosomal abnormalities, mutagenicity, genotoxic potential

1. INTRODUCTION

Tremendous benefits have been derived from the use of pesticides in forestry, public health and the domestic sphere – and, of course, in agriculture. Pesticides have been an integral part of the process of increasing crop productivity, by reducing losses from the weeds, diseases and insect pests that can markedly reduce the amount of harvestable produce. Webster et al. (1999) stated that "considerable economic losses" would be suffered without pesticide use, and quantified the significant increases in yield and economic margin that result from pesticide use. Moreover, in the environment most pesticides undergo photochemical transformation to produce metabolites which are relatively non-toxic to both human beings and the environment (Kole *et al*., 1999).

In order to improve agricultural yield through elimination of diseases and pathogens of crops, pesticides are often used indiscriminately, which can have many negative consequences for ecosystems and human health. The increased consumption of pesticides was earlier considered as a good sign of progress in agricultural production, but later the biological magnification of the pesticides into living tissues and the deterioration of ecosystems by the continuous use of pesticides has been observed. It seems that pesticides use in pest and disease control is not a permanent solution. Being under constant chemical pressure, some pests became genetically resistant to pesticides, non-target plants and animals were harmed, and pesticide residues appeared in all areas.

Pesticides, bioactive compounds used in crop protection and food preservation, differ from other chemical substances because they are spread deliberately into the environment. In this context, is an increasing concern regarding the widespread use of pesticides and their potential impacts on the environment and public health. Each pesticide has its own profile, with specific toxicological and ecotoxicological properties. It is a well known fact that the vast majority of plant protection products have a higher or lower toxicity to humans and other mammals, as well as to other groups of non-target terrestrial and aquatic organisms.

Pesticides can contaminate soil, water, turf, and other vegetation. In addition to killing insects or weeds, pesticides can be toxic to a host of other organisms including birds, fish, beneficial insects, and non-target plants.

Insecticides are generally the most acutely toxic class of pesticides, but herbicides can also pose risks to non-target organisms. Pesticide sprays can directly hit non-target vegetation, or can drift or volatilize from the treated area and contaminate air, soil, and non-target plants. Some pesticide drift occurs during every application, even from ground equipment.

Herbicides are designed to kill plants, so it is not surprising that they can injure or kill desirable species if they are applied directly to such plants, or if they drift or volatilise onto them. Many ester-formulation herbicides have been shown to volatilise off treated plants with vapors sufficient to cause severe damage to other plants. Herbicides from the phenoxy group, including 2,4-D, can injure nearby trees and shrubs if they drift or volatilise onto leaves. Exposure to the herbicide glyphosate can severely reduce seed quality. Plants can also suffer indirect consequences of pesticide applications when harm is done to soil microorganisms and beneficial insects.

On the other hand, heavy treatment of soil with pesticides can cause populations of beneficial soil microorganisms to decline. Overuse of chemical fertilizers and pesticides have effects on the soil organisms that are similar to human overuse of antibiotics. Indiscriminate use of chemicals might work for a few years, but after awhile, there aren't enough beneficial soil organisms to hold onto the nutrients. For example, plants depend on a variety of soil microorganisms to transform atmospheric nitrogen into nitrates, which plants can use.

The toxicity of a pesticide depends on the chemical nature of the active substance and of ingredients, dose and concentration of use, as well as the type of formulation. In humans, the pesticide toxicity can occur during the production, conditioning, transport, storage and application thereof or later, the handling or consumption of treated products.

In present, there were 1297 active pesticide substances registered by the European Commission, some of which have been classified as possible or probable mutagens and/or carcinogens by the International Agency for Research on Cancer. Moreover, at 24 May 2013 a restriction on the use of three pesticides belonging to the neonicotinoid family was adopted by the European Commission. These pesticides, clothianidin, imidacloprid and thiametoxam respectively, were identified as being harmful to Europe’s honeybee population (European Commission, Press Release, 2013).

According to a report by World Health Organization (WHO) and United Nations Environment Programme (UNEP), about 26 million pesticide poisoning accidents resulting in approximately 220,000 deaths are recorded annually worldwide (Richter, 2002). It is also appreciated that the pesticides are involved in the development of a variety of diseases. Gu and Tian (2005) estimated that cancer patients resulted from pesticide poisoning account for nearly 10% of the total cancer patients. Chen et al. (2004) found that the incidence of breast cancer was linearly correlated with the frequency of pesticide uses, and organochlorined pesticide, DDT, and its derivative, DDE, is likely responsible for breast cancer (Zhang et al., 2011).

2. METHODS USED FOR MUTAGENIC POTENTIAL STUDIES OF PESTICIDES ON PLANTS

Pesticides which are used in the modern agricultural practices for disease control have some dangerous effects. Higher plants provide valuable genetic assay systems for screening and monitoring environmental pollutants. For this purpose, the *Allium cepa* is one of the most frequently used higher plant species. The *Allium* test for genotoxicity was introduced by LEVAN (1938) and has been used on pesticides in other studies but it has been used by many researchers too, mainly as a bioindicator of environmental pollution testing crude extracts of cyanobacteria as well as to evaluate the genotoxic potential of medicinal plants because this test uses a model that is adequately sensitive to detect innumerous substances that cause chromosomal alterations.

The *Allium cepa* test is important since it is an excellent model *in vivo*, where the roots grow in direct contact with the substance of interest (i.e. effluent or complex medicinal mix being tested) enabling possible damage to the DNA of eukaryotes to be predicted. The analysis of chromosomal alterations can be equal to the test of mutagenicity mainly for the detection of structural alterations;

however, it is possible to observe numerical chromosomal alterations, as well. The *Allium cepa* test is one of the few direct methods for measuring damage in systems that are exposed to mutagens or potential carcinogens, and enables the evaluation of the effects of these damages through the observation of chromosomal alterations. It is advantageous to use the *Allium cepa* test system since its main component is a vascular plant, making it an excellent genetic model for evaluating environmental pollutants, detecting mutagens in different environments and evaluating many genetic endpoints (point mutations to chromosomal alterations). Relevant studies by Fiskesjö (1985), showed the importance of the *Allium cepa* test system for evaluating genotoxicity, demonstrating that *Allium cepa* cells contain an oxidase enzyme system capable of metabolizing polycyclic hydrocarbonates.

Even though other test systems have been shown to be sensitive for this detection, the results of the *Allium cepa* test should be considered as an alert for other organisms (i.e. bioindicators). The principle of *Allium* test method can be also applied on other plants (generally with a small number of large chromosomes) for studying the mutagenic potential of pesticides.

3. MUTAGENIC POTENTIAL OF PESTICIDES ON PLANTS

Mutagenic effects of herbicides

A large number of studies have shown that some herbicides acts as mutagenic agents. Thus, the investigation carried out by Yuzbasioglu *et al*. (2003) in order to study the cytological effects of the herbicide Racer "flurochloridone" (3-chloro-4-(chloromethyl)-1-[3-(trifluoromethyl)-phenyl]-2-pyrrolidinone) on *Allium cepa* with respect to the cell response, mitotic index, mitotic abnormalities and chromosome aberrations, showed that the concentrations of 80 ppm (LD50), 40 ppm (LD50/2) and 20 ppm (LD50/4) induced a significant amount of abnormalities in mitosis. Their results indicated that flurochloridone herbicide reduced the mitotic division in *Allium cepa* as compared to their control groups. Various types of abnormalities were found into the meristematic root-tip cells (Fig. 1), including C-metaphase, laggards, stickiness, bridges, fragments, multipolarity and polyploidy, micronucleated cells were also observed at interphase. In addition, chromosome breaks, fragments and sister chromatid union were detected in the treated root tips. The percentage of total abnormalities gradually increased with the duration of treatment.

By using a *Helianthus annuus* plant test system, Inceer *et al*. (2004) investigated the effects of the herbicide linuron on somatic chromosomes. The observations carried out on meristematic root cells after treatments with 30, 120, 240, and 500 ppm concentration of linuron for 3, 6, and 12 hours, showed that mitotic index significantly reduced as the concentration was increased and the period of treatment was prolonged. In most cases, the percentages of abnormal mitotic phases were seen to increase with increasing the concentration It was found that linuron had a marked mitodepressive action on mitosis. The types of chromosomal abnormalities observed included fragments, disturbed metaphase, c-mitosis, lagging chromosome, and chromatid bridge. The most common types of abnormality observed were fragments, lagging chromosomes, and chromatid bridges, suggesting that linuron may have genotoxic effects in higher plants. However, the frequencies of these chromosomal abnormalities were not related to concentration and treatment periods. It was found that C-mitosis (c-metaphase) and lagging chromosomes were less common types of chromosomal abnormalities. The observations carried out by Inceer *et al*. (2004) revealed also that the frequencies of these chromosomal abnormalities increased after treating the root tip cells for longer periods (6 and 12 hours).

The cytogenetic effects of the herbicide Avenoxan (active substance 2,4-D) were investigated in both *Allium cepa* and *Allium sativum* (Gul *et al*., 2006) plant test systems. All of the concentrations of Avenoxan used in this study significantly induced abnormalities such as c-Mitosis, chromosome stickiness, bridges, laggards, multipolar cells, compared to control. Also, Avenoxan significantly decreased mitotic index. Inhibition of the mitotic index was dependent on the concentration (0,1%, 0,2% or 0,4%) and time of treatment (3, 6 or 12 hours, respectively). Similar results were obtained

in both *Allium cepa* and *Allium sativum*. The highest abnormality number was observed in root tips of *Allium sativum* for the 0,2% concentration and a 6 hr treatment period, while the highest number of abnormalities was seen in root tips of *Allium cepa* for the 4% concentration and a 6 hr treatment period. The most frequent abnormalities were seen at metaphase, and at this stage the predominant type of abnormality was c-Mitosis.

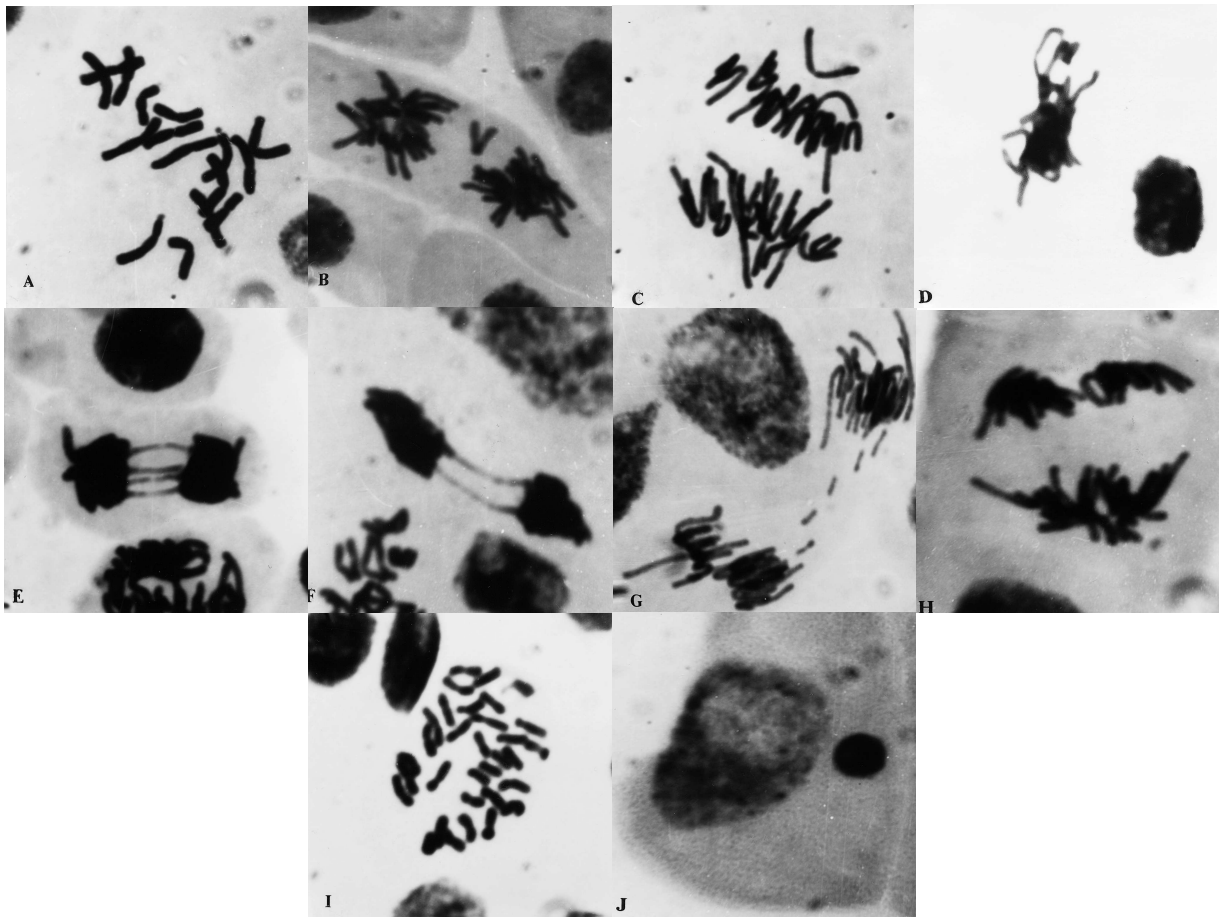

Figure 1. Different types of aberrations induced by the flurochloridone herbicide in Allium cepa root tips. A) C-metaphase; B) Lagging chromosome at ana-telophase; C) Lagging chromosome at anaphase; D) Sticky metaphase and fragment; E) Multi-bridges at telophase; F) Double-bridges at telophase; G) Fragments at anaphase; H) Multipolar anaphase; I) Polyploidy; J) Micronucleus at interphase (Yuzbasioglu et al., 2003).

The genotoxic potential of the commercial herbicide Illoxan (containing 284 g/L diclofop-methyl) was determined by Yuzbasioglu *et al*. (2009) by studying chromosome aberrations in *Allium cepa* root tip cells treated with 37.50, 75.00, and 150.00 mg/L concentrations for 12, 24, and 48 hours. Their results indicated that Illoxan significantly increased the abnormal cell frequency at all concentrations and treatment periods, when compared with controls, and this increase was dose-dependent for the 24 and 48 h treatments. It was found also that Illoxan significantly decreased the mitotic index (MI) in all treatments, when compared with their controls. The decrease in the mitotic index was slightly dose-dependent for the 24 and 48 h treatments. Illoxan did not affect the percentage of mitotic stages, but several types of aberrations were recorded: stickiness (the most common abnormality), laggards, C-mitosis, bridges, chromatid breaks, multipolarity, and fragments. Although they were not significant when compared to the controls, aberrations such as micronucleated and bi-nucleated cells at interphase were induced also by the herbicide Illoxan. Yuzbasioglu *et al*. (2009) reported also that Illoxan can induce polyploidy in *Allium cepa*.
The experiments carried out with the Agil herbicide (Bonciu, 2012) using concentrations of 0.5 ppm, 1.0 ppm and 1.5 ppm for 8 h, 24 h and 48 hours, have shown that the mitotic index in *Allium* root

cells decreased with increasing the herbicide concentration, at each exposure time. Also, the higher concentration and the higher exposure time of the Agil herbicide caused an increase of prophase frequency and decrease of telophase frequency. The frequency of the chromosomal aberration was markedly higher at 1.5 ppm, compared to other test concentrations.

The results indicate that Agil herbicide has the ability to cause production of some mitotic abnormalities (anaphase bridges, binucleated cells). These abnormalities appeared in varying degrees, depending on the herbicide concentration. Also, this study revealed a direct correlation between herbicide concentration, exposure time, and mutagenic effects observed in exposed *Allium cepa* cells.

Recently, the cytogenetic effects of herbicides Atrazine, Avenoxan, Diuron, and Quizalofop-P-ethyl (QPE), were evaluated in the root tip meristem cells of *Allium cepa* by Sharma and Vig (2012). The effective concentration value was determined as approximately 0.5 ppm in the case of Atrazine and Avenoxan, and 1.0 ppm in the case of Diuron and QPE herbicides. Mitotic index decreased with increasing herbicide concentration at each exposure time (6, 12, and 24 hours). In ana-telophase cells, the total percentages of different chromosomal aberration like stikiness, bridges, break(s), ring chromosomes, vagrant chromosomes, c-mitosis, delayed anaphase, laggard(s) and micronuclei at high concentration (1 ppm) were calculated as 31.85% (Atrazine), 29.94% (Avenoxan), 36.66% (Diuron) and 41.04% (QPE). The total number of chromosomes aberrations increased as herbicide concentration increased. Micronucleated cells were also observed at different stages of the cell cycle. The frequency of the micronucleus was markedly higher at 1 ppm than at other test concentrations.

Mutagenic effects of fungicides

A relatively large number of chemicals used as active ingredients in commercial fungicides have been proven to act as mutagenic agents. Thus, based on the results of their studies, Barakat *et al*. (2010) reported that the various treatments with mancozeb have had a marked reducing effect on mitotic index values of *Allium cepa* meristematic root cells, as compared with the control. The mitotic index values progressively decreased as the concentration of the fungicide and the period of treatment increased. The mitotic index reached a minimum value of 1.60% and 2.54% after 48 hours treatments with the concentrations 1.25 and 0.625 mg/L of mancozeb. Based on the inhibitory effect of the fungicide mancozeb on the cell cycle, they concluded that the reduction in mitotic activity may be due to arrest of mitotic cycle at the G_2 phase and/or the prolonged duration of S-phase, but not to inhibiting the DNA synthesis. At the cytological level, mancozeb caused reduction in mitotic index and induced a number of chromosomal abnormalities like stickiness, c-metaphase (2n), c-metaphase (4n), laggards, disturbed metaphase, disturbed anaphase-telophase, multipolar anaphase-telophase.

Mutagenic effects of insecticides

Compared to herbicides and fungicides, much more chemicals used as active ingredients in commercial insecticides have been proven to act as mutagenic agents. The following examples are only a few from the large number of insecticides which can act as mutagenic and genotoxic agents for the genetic material of both plant and animal cells.

Based on their results from investigations with many insecticides, Asita and Matebesi (2010), reported that treatments of *Allium cepa* meristematic root-tip cells for 24 hours with Hormoban, Storm killer, Villa, Fungi-nil, Bexadust, Aphicide and Karbadust were genotoxic. For the pesticides that induced genotoxic effects, the c-anaphase and stick chromosomes classes made up 75% and above, of the total chromosome aberrations, with the exception of one dose each of Storm killer and Villa, where the c-anaphase and stick chromosomes classes made up 50% of the total chromosome aberrations observed. The most common types of aberrations observed were therefore c-anaphase and stick chromosomes. Hormoban, Storm killer and Villa induced bridges $\geq$ twice the control value at one dose each. The formation of chromosomal bridges was not accompanied by the

occurrence of chromosomal fragment. Only Bexadust and Karbadust induced multipolar anaphases and telophases.

Moreover, the mitotic indices of onion root tips treated with all the investigated pesticides were reduced to half or less than half compared with the negative control at one or more doses.

The genotoxic effects of Ethion were reported by Lamsal *et al*. (2010), who investigated the mitotic cell division of *Allium cepa* meristematic roots treated with various concentrations (25, 50, 75, and 100%) of ethion solutions for different duration of time. Their result revealed that increase in the concentration and duration of treatment decreases the mitotic indices. A 24 hours treatment at 100% concentration of ethion induced lowest mitotic index, as compared to that of the control. The percentage of chromosomal abnormalities in different mitotic stages was significantly higher than that of the control, in all the treatment periods and concentrations. These abnormalities reached a maximum value after 12 h of treatment at 75% concentration, and included scattered prophase, non-synchronized condensation of chromosome, disturbed prophase, equatorial plate shifting, sticky chromosomes, C-metaphase, and sticky metaphase.

Recently, Ananthakrishnan *et al*. (2013) investigated the cytotoxic and genotoxic effect of pesticides Furadan and Enodosulphan by using *Allium cepa* test system. The meristematic root cells of *Allium cepa* treated with Furadan in concentrations of 50 and 100 µg/ml for 6 and 24 hrs, respectively, showed various types of chromosomal aberrations (Fig. 2), such as multi-polar anaphase, telomere puffing, disorted metaphase, metaphase clumping, laggards, strap nucleus, strap nucleus with micronucleus, anaphase bridge, telophase with one end puffing, C-metaphase, mature cell puffing and micronucleus. Similarly, the root tip cells of *Allium cepa* treated with Enodosulphan concentrations for 6 to 24 hours showed a large range of chromosomal aberrations, such as strap nucleus, telomere puffing, scattered metaphase or disoriented metaphase, metaphase clumping, multipolar anaphase, laggards, star anaphase, anaphase bridge, nuclear lesions, bi-nucleate cell, nucleoids, tropokinesis, cell showing one end anaphase stage and another end uncoiled telophase puffing. Their results revealed also that both of the tested pesticides are mito-depressive, as the mitotic index of both Furadan and Enodosulphan treated root tip cells showed significant decrease in 50 and 100µg/ml concentration for 6 and 24 hrs.

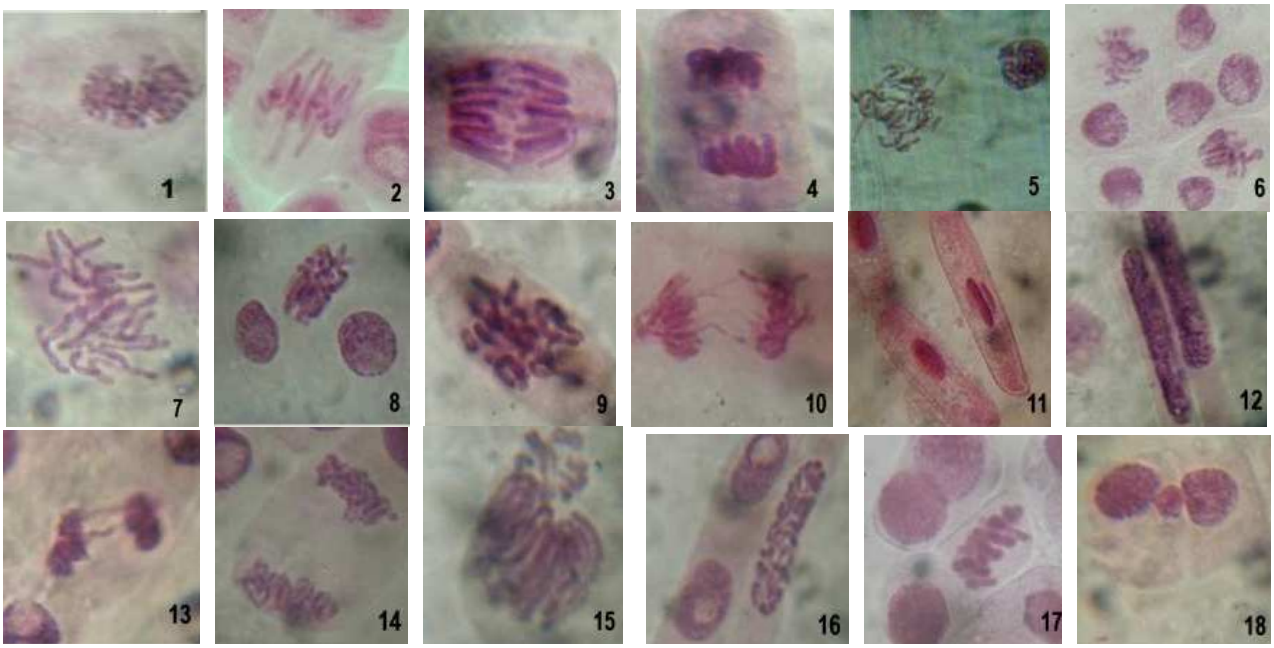

Figure 2. Stages of cell division: 1. Prophase; 2. Metaphase; 3. Anaphase; 4. Telophase. Types of aberrations: 5. Multipolar anaphase; 6. Telomere puffing; 7. Disoriented metaphase; 8. Late prophase showing puffing and stickness; 9. Metaphase sticking; 10. Laggards; 11. Strap nucleous with micronucleus; 12. Strap nucleous; 13. Anaphase bridge; 14. Telophase with one end puffing; 15 C-metaphase; 16. Mature cell division showing puffing; 17. High degree of clumping; 18. Micronucleus (Ananthakrishnan et al., 2013).

4. CONCLUSIONS

We depend on plants for food and other consumer products. Huge amounts of money are spent every year to grow and harvest plants. To maintain healthy plants and to increase yield, many people use pesticides to control weeds, insects, and plant diseases. While pesticides can be important tools, using an integrated approach can be helpful in achieving long-term control of the pest. On the other hand, most pesticides are very toxic (mutagenic, genotoxic, or even carcinogenic) to living organisms and they can easily contaminate the environment (water, soil), thus entering the food chain easily and rapidly.

The studies that have been presented in this review show that many different pesticides tested on *Allium cepa* have major cytological and cytogenetic effects on these plants. Without ignoring their significant beneficial effects, they should be seen as highly polluting chemicals, which can induce mutagenic and genotoxic effects in living cells.

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