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ENVIRONMENTAL TOXICOLOGY AND ANALYSIS OF IMPACT OF PESTICIDES ON *LENS CULINARIS*

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ABSTRACT

BIOMETRICAL GENETICS" has gained wide application in cytotaxonomy of plants in recent years. The new agricultural practices and crop management techniques make it obligatory to use pesticides and insecticides by the farmers. The pesticides are being used by the agricultural scientists for the protection of cultivated plants frequently and extensively. The use of pesticides in high concentration interferes with the infrastructure of chromosomes and their behavioral pattern. Its indiscriminate use has dangerous effect on human being and another living organism.

The present study is regarding impact of pesticides on *Lens culinaris* and environmental toxicology. *Lens culinaris*, a leguminous plant and a member of pulse group. The use of pesticides such as- Monocrotophos, Endocel and Dimecron attributed to the toxic effects like inhibition of cell division, the increase in seedling mortality due to the disturbances at biochemical and cytochemical levels, which in turn cause chromosomal aberration, formation of laggards in the chromosome and extra-chromosomal injury.

The use of pesticides has toxic effect on environment also. By using it we are not only getting rid of the pests but also polluting the soil. It kills micro-organism present in soil and contaminate the underground water, which is dangerous for human health. In Punjab, it has been reported that indiscriminate use of pesticides caused cancer in large number of farmers. My present work also focuses on cytogenetic and mutational studies based on laboratory and field research.

KEYWORDS: Biometric Genetics, Cytotaxonomy, *Lens Culinaris*, Monocrotophos, Endocel, Dimecron, Cytochemical, Cytogenetic, Chromosomal aberration, Laggard.

INTRODUCTION

The trend of *Lens culinaris*, a leguminous plant and a member of pulse group, is a major source of protein, which is the most essential requirement of life. The present study is regarding impact of pesticides on *Lens culinaris* and environmental toxicology. Pesticides are used by the farmers frequently and extensively to get rid of pests and protect the cultivated plants. However, it interferes with the infrastructure of chromosomes and their behavioral pattern. Also, it has toxic effect on the living being and on entire eco- system.

In contemporary period "biometrical genetics" has been widely applicable in cytotaxonomy of plants. It has been possible to split a large number of varieties belonging to the same species into different clusters with the help of 'Karyotypic analysis' and 'Mahalanobis generalized distance value'. Various researchers like (White, 1954) and (Stebbins, 1971) have also shown that the chromosomes are not merely assembly of genes into groups controlling the developmental processes but are of paramount importance as regards to the process of

evolution. The process of evolution is entirely dependent upon variation and selection. The variability can be spontaneous or it may be introduced with the help of invisible rays, chemicals and any other potent toxic substance. The chemicals as a potent tool for inducing mutation was confirmed during second world-war, the credit goes to Aurbach(1943), Ochlara(1943) and (Rapaport 1946), who were the pioneers in the field of chemical mutagenesis. Like other potent mutagens, these chemicals are capable of destabilizing the morphology of chromosomes and are capable of rearranging the genes which may lead to the origin of new varieties and species.

The new techniques in agricultural practices make it obligatory for the farmers to use pesticides and insecticides. The indiscriminate use of such chemicals with toxic effects have dangerous potent probabilities for the consumers. Keeping this in view, many researchers like Ahmad and Grant (1972) Mohan (1975), Reddy and Rao (1981) and others have reported deleterious effects for various pesticides and insecticides on chromosomes. The use of these chemicals in high concentrations does interfere with the infrastructure of chromosomes and their behavioral pattern. Therefore, in the present studies efforts have been made to survey the deleterious effects of pesticides on genetic system and their consequences in the subsequent generations. Chemicals in general are capable of substituting H- atom from a molecule and act directly on D.N.A. infrastructure.

Lens culinaris, a member of pulse group, is a major source of protein. The legume seed proteins are composed of albumin, globulin, glutelin and prolamin sub-fractions. Most of the enzymes are concentrated in albumin (Danielson, 1956), whereas globulin is considered to be the storage protein (Boulteret.al 1967). It is envisaged that the alteration in the ratio of globulin/ albumin will bring out proportionate change in amino acid composition and ultimately the quality of seed protein (Brohult and Sandegren 1954). The efficacy of various mutagenic agents through mutation breeding has not only been illustrated for quantitative character (Gottschalk and Muller 1970, Sinha and Godward 1972, Choulwaret .al 1986, Grinoet al. 1987, and many others), but also for qualitative characters (Prasad and Das 1981, Gottschalk and Wolf 1983).

In the present investigation efforts have been made to find out the effects of pesticides on the chromosomes and its consequences in subsequent generations and biochemical studies have been undertaken in control as well as mutated populations. The results obtained give interesting conclusions, which may be pursued further. It has now been fully established that studies of Karyotypes have led to the way to a new and fuller understanding evolutionary tendencies within major group of plants and a potent aid to the complete reorganization of the taxonomic system of the group.

It has helped in identifying the trends of evolution and establishing the missing links. It has been considered by various workers such as White (1946), Stebbins (1951) and others that are not only structures that result as and products of a series of gene controlled developmental processes but they are the bearer of hereditary characters also. The structural organization and gene controlled functions of the chromosomes might have evolved via process of mutational changes and natural selection because of their adaptive potentialities in the population to which they belong. In many instances, differences between populations with respect to chromosomal structures and their final composition could be used to identify the different ways of becoming adapted to similar situations. Considering the above facts endeavours have been made to investigate the cytotoxic differences in different varieties of *Lens culinaris*. The pesticides (monocrotophos, endocel and dimecron) are being used by the Agricultural Scientists for the protection of cultivated plants frequently and extensively.

The active ingredient in pesticides under reference might be toxic to the basic infrastructure. In the present investigation, efforts have been made to find out their toxic effect on mitotic and meiotic behaviour as well as polygenic traits. The different parameters used to identify the deleterious effects are being reported in terms of germination and survival percentage, frequency of chlorophyll mutation percentage, and types of mitotic and meiotic aberrations, pollen sterility and polygenic variation with special reference to height of the plant, number of branches, flowering days, number of pods and seeds and seed weight.

The Biochemical Analysis of the mutants and its comparison with the controls which gives a definite proof of changes entered at molecular level due to the effects of pesticides at different concentrations. The

significant increase in Amino-Acids, Total soluble seed proteins and its Sub-fractions was encountered at lower dose-levels in all the varieties. However, the higher doses caused significant reduction. The ratio of Globulin, Albumin has been apparently changed in treated populations. In all the varieties which reflects the qualitative as well as quantitative changes in the proteins). Such findings can be utilized for selection purposes and it may be a supporting tool to the cytologist in their breeding programme for evolving better varieties with greater nutritional value.

The Electrophoretograms indicated that the proteins are separated mainly according to their molecular weight. This is because S.D.S. binds to the proteins giving them large number of negative charges due to the sulphates. All the proteins ultimately migrate according to their size. The large proteins move more slowly than the smaller ones because they encounter more resistance when traversing the molecular codes within the polyacrylamide gel used for electrophoresis. The higher value of percentage of most common bands (POMCOMB) indicates a high degree of retention of common characteristics in the electrophoretograms. In the electrophoresis, the POMCOMB values for intra-varietal comparison in controls have been found to be higher than the values for inter-varietal comparisons. The POMCOMB value are sufficiently lower for treated population at higher dose levels indicating mutation induced damages.

MATERIAL AND METHODS

The material used here was *Lens Culinaris* obtained from Rajendra Agricultural University, Pusa (Bihar). The seeds of all the varieties of *Lens culinaris* were soaked in water and were then allowed to germinate in separate Petri dishes on moist cotton and filter paper at $21^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The seedlings were transferred to earthen pots filled with soil and low proportion of sand for porosity. The plants were kept under natural conditions. Floral buds of different sizes were collected randomly and were fixed in aceto-alcohol (1:3) containing a drop of ferric chloride for 24 hrs. It was further transferred in 70% alcohol for preservation.

The Pesticides used in the research program namely:

1. Monocrotophos,
2. Endocel and
3. Dimecron were obtained from the various commercial sources

The morphological characters were studied by visiting the experimental field regularly. Data's were collected on variants such as: (i) Early maturity, (ii) Flowering time, (iii) Sterility and (iv) Seed characters etc. and for the evaluation of M1 and M2 generations, the following parameters were taken into consideration:

- a. Percentage of germination.
- b. Percentage of survival
- c. Height of the mature plant
- d. Number of Branches.
- e. Days taken for first flowering (After how many days it flowered).
- f. Number of pods per plant and
- g. Weight of 100 (hundred) seeds.

During the course of present research program, endeavors were made to look into the details of the mutagenic effects of three chemicals (all pesticides) Monocrotophos, Endocel and Dimecron on some biological parameters of *Lens Culinaris*. My work also focuses on cytogenetic and mutational studies based on laboratory and field research.

The parameters studied were:

- (a) Percentage of germination
- (b) Time taken for germination
- (c) Seedling survival
- (d) Percentage of mitotic and meiotic aberrations
- (e) Effects on plant attributes and

(f) Chlorophyll mutation.

RESULTS

Germination

1. The percentage of germination reduced immediately after the treatment of monocrotophos, endocel and dimecron.
2. As the concentration of the chemical solution was increased, there was a gradual decrease in the percentage of germination in all the varieties taken.
3. The percentage of seedling survival in this case was inversely proportional to the concentration of the chemicals applied.

The effects of pesticides on percentage of germination as well as on percentage of survival of seedlings in *Lens culinaris* M1 and M2 generations are illustrated in graphs- 1a, 1b, 2a and 2b. Also, the mitotic and meiotic aberrations induced by pesticides are shown in plate-4 and plate-5.

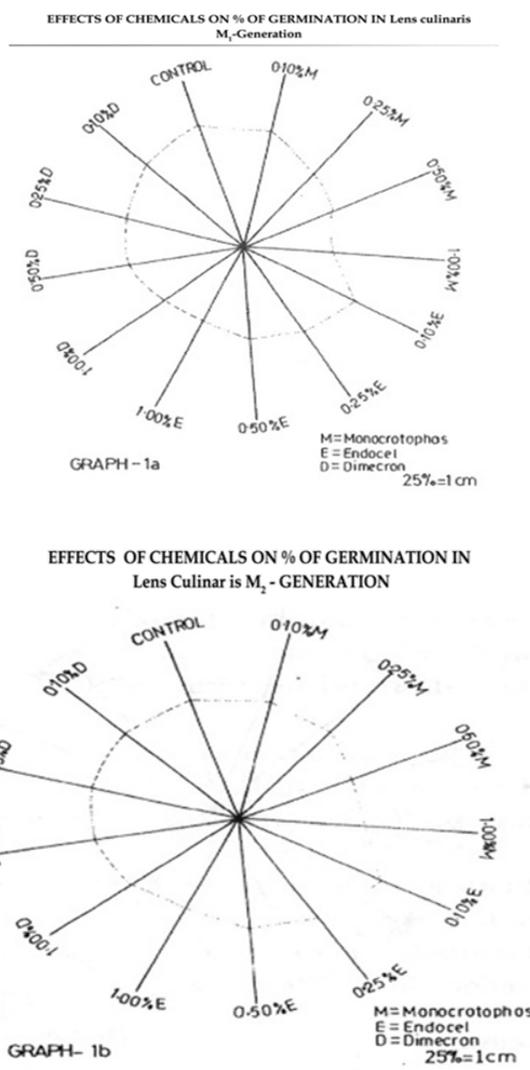


Figure 1. (a) Effects of Chemicals on % of Germination in *Lens Culinaris* M1 -Generation
 (b). Effects of Chemicals on % of Germination in *Lens Culinaris* M2 -Generation
 Source: Singh, 2014.

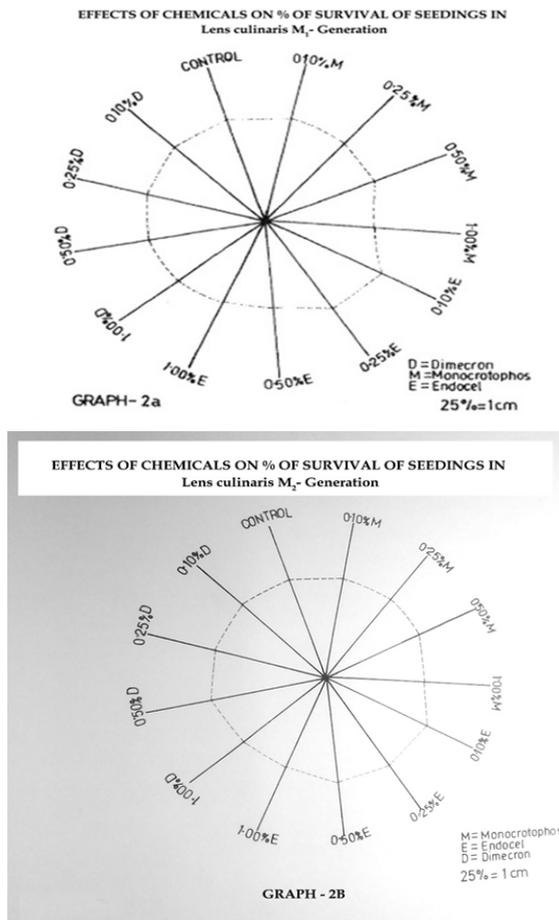


Figure: 2(a). Effects of Chemicals in % of survival of Seedings in *Lens Culinaris* M1 - Genration ; 2(b). Effects of Chemicals in % of survival of Seedings in *Lens Culinaris* M2– Genration
Source: Singh, 2014.

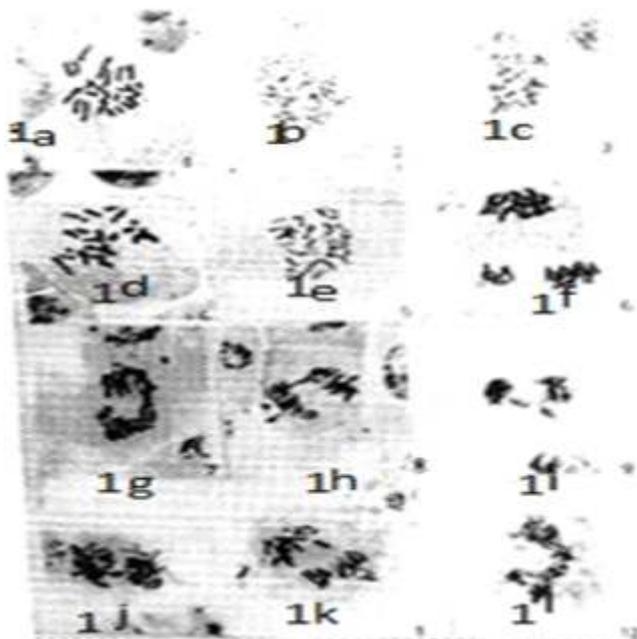
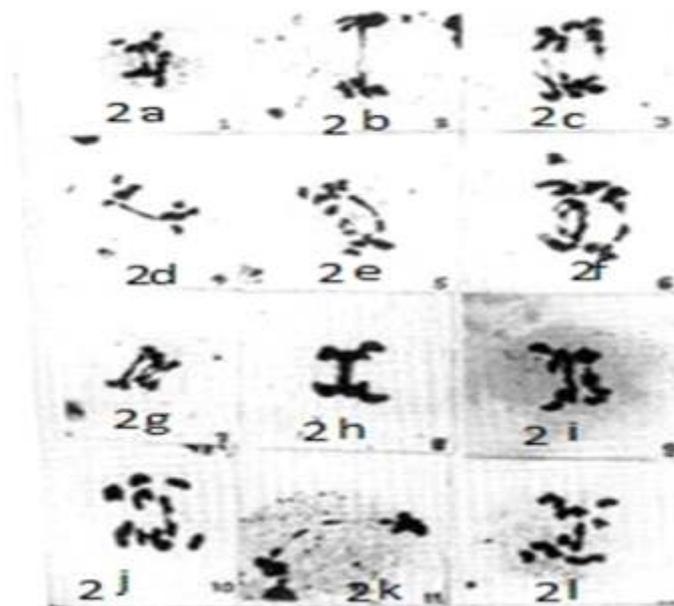


Plate 1: Mitotic aberrations induced by Monocrotophos Endocel and Dirnecron in *Lens Culinaris*

- 1a. A polyploid cell (1.00% E)
 1b. A polyploid cell (1.00% E)
 1c. A polyploid cell (0.50% E)
 1d. Chromosomes at Metaphase showing multiple breaks and possible formation of microfragments (1.00% M)
 1e. A polyploid cell (1.00%)
 1f. Tripolar Anaphase cell (0.50% M)
 1g. Anaphase showing Single Bridge (1.00% D)
 1h. Anaphase showing Single Bridge (0.50% D)
 1i. Anaphase showing Single Bridge (0.50% E)
 1j. Anaphase showing Single Bridge (0.10% E)
 1k. Anaphase showing Lagging chromosome (0.25% M)
 1l. Anaphase showing un-orientation of chromosomes (1.00% M)
 Source: Singh, 2014.

Plate: 2. Meiotic aberration induced by Monocrotophos Endocel and Dimecron in *Lens culinaris*

- 2a. Anaphase 1 showing single bridge (0.25% E)
 2b. Anaphase 1 showing single bridge (0.50% D)
 2c. Anaphase 1 showing single bridge (1.00% E)
 2d. Anaphase 1 showing single bridge (1.00% E)
 2e. Anaphase 1 showing single bridge with laggards (0.50% E)
 2f. Anaphase 1 showing single bridge (1.00% M)
 2g. Anaphase 1 showing single bridge (0.50% M)
 2h. Anaphase 1 showing single bridge (1.00% E)
 2i. Anaphase 1 showing single bridge (0.50% D)
 2j. Anaphase 1 showing single bridge (0.10% M)
 2k. Anaphase 1 showing single bridge (0.25% E)
 2l. Anaphase 1 showing single bridge (0.50% D)
 Source: Singh, 2014.

In the present study, there existed an inverse relationship between the percentage of survivors and chemical concentration. Decrease in survivors due to chemical treatments was attributed to the toxic effects of chemicals like inhibition of cell division. The increase in seedling mortality was also known to occur due to the disturbances at biochemical and cytochemical levels, which in turn caused chromosomal and extra-chromosomal injury. The occurrence of decreased survival at maturity was suggestive of the fact that the effects of chemicals were detrimental to the mechanics of germination as well as survival. It seemed that despite gross injury sustained at physiological and cytological level, the cells were capable of undergoing mitotic divisions to a certain extent along with the normal or apparently normal cells.

The trend of decrease in the percentage of survivors at maturity as compared with the germinated seedlings could also be explained on the basis that after seedling stage these plants required more nourishment and cytological activities for their normal growth and development. But, the presence of damaged cells in abundance made them incapable of undertaking all essential vital functions and thus they were relegated to the position of cytological and physiologically crippled, which ultimately resulted in their death before reaching the stage of maturity. The decrease in the percentage of survival was also attributed to the destruction of auxins.

CONCLUDING REMARKS

As we know nitrogen fixation is done by nitrogen fixing bacteria (*Rhizobium*) and it is very much present in the soil, so if the soil is polluted, it will be suffocating for the micro-organisms and they will die. The adverse effect is seen in the plants genetic recombination also. The use of pesticides such as- monocrotophos, endocel and dimecron attributed to the toxic effects like inhibition of cell division, the increase in seedling mortality due to the disturbances at biochemical and cytochemical levels, which in turn caused chromosomal aberration, formation of laggards in the chromosome and extra-chromosomal injury.

Humans and micro-organism have a common interest: the soil. To earthworms and multiple other organisms, the soil is home and a food source. For humans, too, the soil is a source of food, through production of edible crops or crops for livestock feeding. Pesticide use may influence the soil negatively, if key functions are disturbed. By using pesticides we are not only getting rid of the pests but we are polluting the soil too. The habitat of the micro-organisms present in the top soil is being destructed. So, they are losing lives, and on the other hand the most fertile layer of the soil is being lost resulting into soil degradation. Rama Rao (1962) called this very situation as the creeping death of the soil. It has disturbing effects on entire eco-system.

Risk assessment of pesticides is therefore necessary, but should take into account the specific application. Management practices other than pesticide application also influence soil organisms and their activity. Interactions between management practices should be taken into account when evaluating pesticide effects, the report concludes. It is recommended that evaluation of pesticides in test systems should also consider factors other than the direct exposure to a pesticide in connection with risk assessment.

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