CYTOTOXIC AND CLASTOGENIC EFFECTS OF SOME INSECTICIDES IN *Allium cepa* L.

By

JAYAPRAKASH NAIK. B

THESIS

Submitted in partial fulfilment of
the requirement for the Degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University

Department of Agricultural Botany
COLLEGE OF HORTICULTURE
Vellanikkara, Trichur - 680 654
KERALA INDIA

1983

DECLARATION

I hereby declare that this thesis entitled "Cytotoxic and clastogenic effects of some insecticides in *Allium cepa*, L." is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title, of any other University or Society.

Vellanikkara,
19- 11 - 1983.

Jayaprakash Naik, B.

Dr.N.K. Vijayakumar,
Associate Professor
(Genetics).

Regional Agricultural
Research Station,
Kerala Agricultural University,
Pilicode.

Dated: 19-11-1983.

CERTIFICATE

Certified that this thesis entitled "Cytotoxic and clastogenic effects of some insecticides in *Allium cepa*, L." is a record of research work done independently by Sri. Jayaprakash Naik, B., under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

N.K. Vijayakumar,
Chairman,
Advisory Committee.

CERTIFICATE

We, the undersigned, members of the Advisory Committee of Sri. Jayaprakash Naik, B., a candidate for the degree of Master of Science in Agriculture with major in Agricultural Botany, agree that the thesis entitled "Cytotoxic and clastogenic effects of some insecticides in *Allium cepa*, L." may be submitted by Sri. Jayaprakash Naik, B. in partial fulfilment of the requirement for the degree.

Chairman :

Dr. N.K. Vijayakumar

Members :

Dr. K.M. Narayanan Namboodiri

Smt. Acchamma Oommen

Dr. P.J. Joy

ACKNOWLEDGEMENT

It gives me immense pleasure to express my deep sense of gratitude and indebtedness to my major advisor, Dr. N.K. Vijayakumar, Associate Professor (Genetics) for suggesting the problem and for his expert guidance, keen interest, inspiration, constructive criticism and sustaining encouragement throughout the course of this investigation. I count it as one of the most precious experiences of my life to have been able to work under his guidance.

I take this opportunity to express my profound thanks and gratitude to Dr.K.M. Narayanan Namboodiri, Professor and Head, Department of Agricultural Botany, for his constant inspiration and helpful suggestions throughout the course of this study.

My sincere and heartful thanks are also due to Dr. P.J. Joy, Associate Professor (Entomology) and Smt. Acchamma Oommen, Assistant Professor (Agricultural Botany), the members of my advisory committee for being available with their helpful suggestions and valuable advice.

I am grateful to Dr. C.C. Abraham, Associate Director of Research for envincing keen interest in this

study and for arranging the insecticides required for the study. The Rallis India Ltd., Bombay and National Organic Chemical Industries Ltd., Bombay are gratefully acknowledged for providing the insecticides for this study.

As a special note, I express my thanks to Dr.P.K. Gopalakrishnan, Associate Dean for providing the necessary facilities for the conduct of this study.

I have a special word of thanks and sincerest appreciation to Mr.K. Madhavan Nair, Associate Professor (Instrumentation) for lending a helping hand for taking photomicrographs.

My sincere thanks are also due to my teachers and staff members of Department of Agricultural Botany for their whole-hearted co-operation and assistance which enabled me to complete this investigation successfully. I express my whole-hearted gratitude to all my friends, especially, Sri. L. Rajamony and Smt. A. Naseema for their timely and manifold help.

The Junior Fellowship received from the Kerala Agricultural University during the course of my study is gratefully acknowledged.

On a personal note, I am greatly indebted to my esteemed uncle, parents and sisters for their constant encouragement and good wishes.

Jayaprakash Naik, B.

TO

MY BELOVED GRANDMOTHER

(SMT. KAVERI R. NAIK)

C O N T E N T S

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

15 Anaphase of *Allium cepa* showing multipolar seggregation of chromosomes.

16 Telophase of *Allium cepa* showing persisting chromosome bridge.

17 Telophase of *Allium cepa* showing broken chromosome bridge.

18 Telophase of *Allium cepa* showing lagging chromosome.

19 Telophase of *Allium cepa* showing precocious movement of chromosome.

20 Telophase of *Allium cepa* showing persisting chromatin bridge connections and chromosome fragment.

21 Telophase of *Allium cepa* showing micronucleus along with daughter nuclei.

Introduction

INTRODUCTION

Agriculture production has achieved tremendous progress in the past three decades. The implementation of Green Revolution in general, and newer scientific technology such as use of high yielding varieties, chemical fertilizers, irrigation and mechanised farming in particular, have revolutionised the World agriculture production. It has increased at twice the rate of earlier periods. The advancement in crop production has not been without problems. Pests and diseases are the most important among them and have accounted for 30 to 40 per cent loss of total crop production. Dr. Norman E. Borlaug, the Nobel Luareate, warned once that human life will be doomed by starvation if crop pests are not brought under control. The Indian Agricultural Research Institute has assessed that an annual loss of 20 per cent of agricultural produce valued at Rs.7,000 crores accounts to insect pests alone in India. Today we are conscious of the fact that chemical control have been developed and that poisonous substances are widely used to achieve pest control and reduce crop loss. The consumption of pesticides in India has recorded a tremendous increase for the last two decades. The advantages of pesticides have reached such a level in crop

production, that few zealous farmers who think that if a bit of pesticide is good for their crop, the more the better. This unscientific use and careless handling of pesticides have lead to adverse consequences. Though the use of insecticides is immense, they have limitations too.

An unwarranted danger associated with the indiscriminate use of pesticides is that their toxicity may be exerted in unwanted directions, to the detriment of the user, the consumer and the biological environment in which the chemicals are used. Translocation of these chemicals from their sites of application also occurs through various media of environment (air, water, soil and food). So exposure to pesticides far remote from the site of application, are also possible in the case of persistant insecticides like organochlorine compounds. They are highly stable, causing biological concentration in the food chain, ultimately ending up in human body. The public alarm has been raised against the indiscriminate use of pesticides after Rachel Carson in 1962 dramatically emphasised the dangers to the flora and fauna associated with these poisonous chemicals through her highly laudable book "Silent Spring".

Some of the insecticides besides being toxic, have been known to be teratogenic, carcinogenic and

mutagenic. Mutagenicity of pesticides have been established from studies on micro-organisms (Shirasu, et al., 1976; Carere et al., 1978), plants (Logvinenko and Morgan, 1978), Drosophila melanogaster (Hanna and Dyer, 1975) and mammals (Yoder et al., 1973). The potentially mutagenic insecticides can act with the genetic materials of various organisms coming in contact with them - both target and nontarget - including human beings. Thus, such chemicals present a serious health problem, not only for the present generations through chromosome breakage as well as gene mutations in the somatic cells, but also for future generations through the production of heritable gene mutations and chromosomal abnormalities ultimately leading to a genetic disaster of unpredictable proportions someday in human population. Such an instance has been reported from Illinois, in U.S.A., where John Bask developed an aversion to food prepared from crop produce obtained from growing them using chemical fertilizers and pesticides. The disease is known as "Twentieth Century Syndrome".

Insecticides which are potent mutagens are capable of acting with the genetic material of the pest itself, producing new mutant types. This can lead to the production of new strains of pests which may be resistant to the insecticides used. It was reported that there are 435

pesticide resistant insects all over the world. This number can record prohibitive increase by the use of such chemicals. In addition, a possibility of the break down of the genetic make up of the crop plants also cannot be ruled out, by the repeated application of pesticides which are mutagenic.

Pesticides, thus appear to be a mixed blessing, as on the one hand they help us to increase the quality and quantity of agricultural produce, on the other hand they pollute the atmosphere in which we live. So they can rightly be called a "necessary evil". Therefore, it becomes apparent that all the pesticides should be tested for their various toxicological aspects and above all, their potentiality to act on the genetic material and a balance sheet drawn by comparing the pros and cons and only if the benefits out weigh the risks, their use should be advocated.

Thus, considering that such studies will have far reaching implications, the present investigation was carried out to assess the cytotoxicity and clastogenecity of three insecticides, namely, aldrin, carbofuran and phorate taking Allium cepa, L. as the test material. The objectives of the study are:

(1) to screen the potential cytogenetic effects of these insecticides.

(2) to find out the cytogenetically safe level of each insecticide.

Review of Literature

REVIEW OF LITERATURE

While the value of various agricultural chemicals in controlling unwanted organisms is of unquestionable economic importance, it has only been during the last decade toxicologists have realized that their use has many secondary and unwanted consequences. It is becoming clear now that the hereditory constitution of organisms - both target as well as nontarget - may be changed as a result of exposure to these biocide chemicals by their indiscriminate and careless handling. One such devastating event which attracted the public attention by newspaper headlines was the crippling of a large number of villagers at Hindigodi in North Canara district of Karnataka, as a result of poisoning by parathion and endrin, two potent insecticides widely used in agricultural operations. The unintentional deleterious effects of such chemicals on the environment constitute a prime area of human concern today.

Pesticides comprise one of the most extensively used agricultural chemicals. Exhaustive literature is available regarding the toxicologic, mutagenic and teratogenic effects of various pesticides which are extensively used today. However, an attempt has been made here

to review the cytotoxic, clastogenic and genetic effects of some of the commonly used carbamate, organochlorine and organophosphorus insecticides in various test systems.

A. THE TEST SYSTEMS

In 1975, the Council of Environmental Mutagen Society has recommended a "three tier system" of approach for studying the genetic effects of various environmental chemicals. The test systems included in this approach are:

(a) Microbial system: Yeast (Saccharomyces cerevisiae), bacteria (Escherichia coli), Neurospora etc.
(b) Submammalian system : Drosophila melanogaster.
(c) Mammalian system : in vivo - Laboratory mice (Mus musculus); in vitro - cultured animal and human cells.

However, Grant in 1978 did an elaborate comparative evaluation of the effects of different pesticides and other environmental chemicals in plants and animal materials for chromosome aberrations and mutations and obtained excellent correlation between effects found in root tip systems and mammalian systems. He recommended a group of plants, viz., onion (Allium cepa, L.), broad bean (Vicia faba, L.), barley (Hordeum vulgare, L.), pea (Pisum sativum, L.), maize (Zea mays, L.), soyabean (Glycine max, (L) Merr.), tomato (Lycopersicon esculentum, Mill.),

tobacco (Nicotiana tabacum, L.) etc. to be accepted as a first tier system for the detection of possible genetic damage by environmental chemicals in his report to the Gene-Tox Programme, an Environmental Protection Agency in the United States of America. Same type of correlations were discernible in the results of Behra et al.(1982). Logvinenko and Morgan (1982) have also stressed the need for monitoring mutations in plant systems at least of those used as agricultural chemicals.

Plants were used for studying the induction of chromosomal aberrations from a quite early time, due to its ease and low cost of handling, and amenability to diverse growth and testing conditions. The first cytogenetic study of an agricultural chemical in higher plants dates back to 1931, when Kostoff observed many chromosomal irregularities in mitotic cells and reduced seed set in tobacco and brinjal after the plants had been fumigated with nicotine sulfate (Grant, 1982a). Subsequent studies with many mutagenic chemicals have shown that plants exhibit different types of chromosomal aberrations some of which are specific for different chemicals.

Allium cepa, L. as a classical test system for studying the effects of chemicals on plant chromosomes was developed by Levan in 1938. Shelby (1976) compiled

and tallied the frequency of different plant species used in analysis of chromosome for pesticide toxicity, among 538 references for eight plant species and found that Vicia faba has been used for over a third of the studies (36.4%) and was closely followed by Allium cepa (29%). Grant (1982b) has proposed Allium cepa as an excellant plant species for the assay of chromosomal aberrations after chemical treatments. Protocols also have been given for using root tips from either bulbs or seeds of Allium cepa to study the cytological end points, which help the testing of chemicals in somatic cells.

B. EFFECT OF INSECTICIDES ON DIFFERENT TEST SYSTEMS

I. CHLORINATED HYDROCARBON INSECTICIDES

The synthetic insecticides that had been evolved earlier belonged to chlorinated hydrocarbon group. Due to their persistant and slow biodegradation in nature, they were recommended to be ideal for vector control. Aldrin, dieldrin, chlordane, BHC, and heptachlor are used for seed dressing and soil application to control root attacking pests. Aldrin is used against cockchafer in coconut, and rhizome weevil in banana and BHC controls leaf folder, and casewcrm in rice, rhinocerous beetle in coconut and earhead bug in sorghum. Endrin is used to

control stem borer and trichlorfon controls pests of vegetable crops. These compounds affect the central nervous system and result in nervous derangement, disturbance in the oxidase enzyme system resulting in muscular twitching and failure of respiration. However, their persistance has been the cause of great concern and alarm all over the world due to their adverse effects on nontarget organisms. The ban of DDT, one of the broad spectrum insecticide used once both in public health and agriculture world wide, since 1971 should be remembered in this regard. These insecticides generally cause cytogenetic effects like gene mutations, and chromosome breakage and many secondary effects as reduced fertility.

(a) Plant system

Seeds of barley treated with 500 ppm of Lorox (3-(3,4-dichlorophenyl)-1-methoxy-1-methyl urea) containing 50 per cent active ingredient, for a period of 24 hours showed that all the cells were abnormal in meiotic behaviour. The anomalies found by Wuu and Grant (1966) were stickiness, clumping of chromosomes, chromatin bodies, cytoplasmic furrowing, unequal distribution of chromatin material into daughter cells, and asynchronous and multiple cell division. The abnormalities were similar to the properties of radiomimetic compounds.

Mohandas and Grant in 1972 studied the effects of 2,4-D (2,4-dichlorophenoxy acetic acid) and amitrol (3-amino-1, 2, 4-triazole) in 12 species of plants viz., Tradescantia clone 02, Allium cepa, Vicia faba, Triticum aestivum, Triticum dicoccum, Hordeum vulgare, Secale cereale, Centaurea jacea, Cirsium vulgare, Crysanthemum leucanthemum, Plantago maior and Erigeron canadensis. The cytological abnormalities induced in root tip cells were chromosome bridges, fragmentation, lagging and C-mitosis and albino mutants in M_2 generations in seed treatment. Amer and Ali (1974) compared the effects of 2, 4-D, 2,4,5-T (2, 4, 5-Trichlorophenoxy acetic acid), 2, 4, 5-trichlorophenol and 2,4-dichlorophenol on meiosis, pollen viability and yield of Vicia faba by seed and spray treatment at 15 days and 35 days after germination. The sprayings on 35 days old plants showed higher percentage of abnormal pollen mother cells with stickiness, lagging, fragments and chromatin bodies in it. The spray of 2, 4, 5T on 35 days old plant increased the pollen sterility but the yield was not affected by any of the treatments. In another study on the effect of 2,4-D, picloram (4 amino-3, 5,6-trichloropicolinic acid) and 2,4-D#2, 4,5-T in natural vegetation comprising of Ambrosia artemisiifolia, Pastinaca sativa, Solidago gennalensis, Solidago memoralis

and Vicia cracca, Tomkins and Grant (1976) found a larger proportion of lagging chromosomes and lower proportion of fragmentation than the abnormalities occurring spontaneously. Multipolar spindles were noticed in simazine (2-chloro-4, 6-bis (ehylamino)-1,3,5-triazine) treatment. Shokod'ko et al.(1978) reported the resistance of Praguitis communis to DDT compared to BHC and the concentrations of pesticides had adverse effects on photosynthesis.

The prolonged treatment from 4 to 48 hours of paradichlorobenzene induced contraction and condensation of chromosomes, precocious separation of chromatids, bridges and fragmentation of chromosomes, tetraploid and binucleate cells on somatic cells of Lens esculenta (L) Moench. var. microsperma. The chromosome breaks were restricted to secondary constriction, satellite and heterochromatic regions and rarely on centromere region (Sarbhoy, 1980). When Sharma and Agarwal (1980) treated maize seedlings for three hours with the same chemical as above, it was found to cause accelerated cell division frequency of 50 per cent and induced polyploidy.

The 0.5 mg/ml concentrated spray of monochloroacetic acid (MCA) and trichloroacetic acid (TCA) in Vicia faba at seedling and flowering stages affected pollen mother

cells significantly as observed by Amer and Ali (1980). The abnormalities noticed were lagging, stickiness and fragmentation of chromosomes and pentads at telophase II with TCA treatment. Pollen viability was effected slightly with both the agents.

Bakale et al. (1981) reported that 400 ppm of 2,4-D as lethal to Malvastrum coromandelianum when seeds were treated. They tried other doses as well and found aberrations like bridges, precocious movement of chromosomes, fragments and persistant nucleolus at metaphase. The same results were obtained by Bakale and Kolhe (1981) in somatic cells of Solanum xanthocarpum on seed treatment with sodium arsenite, 2, 4-D and Hyvar x (5-bromo 3-sec-butyl-6-methyl uracil). The rate of division of cells decreased with 2, 4-D treatment. Again in 1981, Bakale and Hadke scored mitotic abnormalities from root tip cells of Euphorbia geniculata (Orteg) grown from the seeds treated with 2, 4-D, Lasso (2-chloro-2, 6-diethyl-N-methoxy-methyl acetanilide) and sodium arsenite for 24 hours. The abnormalities recorded were stray chromosomes, laggards, clumping, grouping and fragmentation of chromosomes. The abnormalities were dose dependant. Sodium arsenite induced highest percentage of abnormalities and Lasso was least effective. The mitotic and meiotic chromosomal aberrations

and chlorophyll deficient mutations were reported by Bhargava and Khalatkar (1981) in diploid barley (Hordeum vulgare) treated with 2, 4-D. They found no pronounced reduction in seedling height, pollen fertility and seedset.

(b) Microbial system

Only very few reports are available on the cytogenetic effects of chlorinated hydrocarbon insecticides. McCoy et al. (1978) reported that the growth of Pol A_1^- (DNA polymerase A defecient) Escherichia coli was inhibited by allyl chloride, a constituent of a number of hydrocarbon pesticides. He concluded that it is an indication of DNA modifying activity of the chemical. A very low toxicity due to 0.01 and 0.10 per cent treatment of lindane in Paramecium primaurelia was observed by Komala (1978). Fallon and Fliermans (1980) found that chlorination of fresh water resulted in nonvolatile mutagenic activity in Salmonella typhimurium TA-100, a nonliver activated system, and concluded that the organic materials of less than 200 molecular weight dissolved in water were responsible for nonvolatile mutagen formation and this activity decayed with a half life of 1 to 5 days. The mutagenic activity in S. typhimurium TA-100 was due to direct action of mutagens as concluded by Maruoka and Yamanka (1980) from

their studies on chlorinated water of lake and river. The ground and distilled water showed no mutagenesis. Leoni et al. (1982) evaluated the hazdards of atrazine (2-chloro-4-ethylamino-6-isoprophylamino-1, 3,5-triazine) chlorobromuran and carbaryl in bacterial system of experiment. Shaw and Garner (1983) in an attempt to test the carcinogenic effects of benzyl chloride, chloromethyl biphenyl and hydroxy methyl biphenyl were tested in Salmonella/microsome and bacterial fluctuation assays, found that all the three chemicals were biologically active in the assays.

(c) Submammalian system

In a study Kramers and Knaap (1975) administered folpet (N-(trichloromethyl thio) phthalimide) and captan (N-(trichloromethyl thio)-4 cyclohexene-1, 2-dicarboximide) by injection into adult males or by feeding to the larvae for mutagenicity testing in Drosophila melanogaster and observed complete and mosaic sex-linked recessive lethal mutations, II - III translocations and dominant lethals.

Vijayakumar et al. (1981) reported that epichlorhydrin (HCH) an important ingredient of many pesticides was a potent genotoxic compound which had different responses in two sexes of D. melanogaster. The females

were more sensitive than the males in respect to LD_{50} values, the doses being 2.2 per cent and 3.1 per cent respectively for female and males.

The toxicity of lindane to various developmental processes of D. melanogaster was studied by Dhingra and Vijayakumar (1981). They observed that lindane was effective as larvicide. The dominant lethal and sex-linked recessive lethal mutations were not increased significantly. However, the fertility of males was significantly reduced at 2.00 and 3.00 ppm.

(d) Mammalian system

(i) Animal system: In one of the studies with 100 to 400 ppm DDT, Johnson and Jalal (1973) reported higher proportions of deletions, stickiness, rings, and metacentric chromosomes in mice than untreated animals indicating mutagenicity of this chemical. In 1975, Mahu and Herbet declared that DDT and DDD were most potent mutagens based on their results of increased frequency of chromosomal gaps and breaks and marked inhibition of mitotic index in chinese hamster cell culture. DDE exhibited weaker influence while DDA neither induced chromosome damage nor did it affect mitotic index. In a cytological analysis, Banerjee et al. (1981) observed that chlordane at the rate of

1 mg/100 g body weight for 10 days induced erosion, stickiness, C-mitosis, gaps and breaks in bone marrow cells of albino rat. The DNA content of brain and lungs increased and that of kidney decreased. RNA content in lungs increased, while in other organs it remained constant. DDT at the dose of 100 mg/kg body weight for 15 days caused only erosion and a few C-mitosis.

Gopalaswamy and Aiyar (1981) reported that the molar ratio of binding of lindane (gamma-HCH) and hexachlorobenzene (HCB) to DNA was of same order as that of dimethyl benzanthracene, a known carcinogen, when they compared these chemicals in the rat liver microsome assay.

Bhojvaid (1980) studied the effects of endosulfan, carbaryl and malathion in albino swiss mice taking mitotic index, chromosomal aberrations and spermhead morphology as the parameters after injecting the insecticide intraperitoneally at the doses 15, 20 and 30 mg/kg body weight with 24 and 48 hours as period of treatments. Bhojvaid and Vijayakumar (1981) found higher concentrations of endosulfan and carbaryl to reduce mitotic index and the effect was pronounced in 48 hours with an increase of 1.2 per cent and 0.8 per cent over control with 20 mg/kg and 30 mg/kg of body weight respectively. The aberrations

recorded were chromatid and chromosome breaks, attenuated chromosomes and polyploid cells.

When the drinking water of mice was incorporated with 5-chlorouracil and a metabolite, 5-chlorodioxy uridine, Pal et al. (1981) noticed that the bases heavily incorporated in liver and testis DNA and sister-chromatid exchange was induced. The 5-chlorodioxy uridine is five times more potent mutagen than 5-bromodioxy uridine in sister chromatid exchange. Vijayakumar et al. (1981) reported various types of chromosome anomalies when they analysed the chromosomes of bone marrow cells and sperm head morphology after treatment with epichlor hydrin. The chromatid and chromosome breaks, centric and chromatid fusion, decondensation, pulverization, polyploidy etc. were recorded. Certain specific abnormalities like diminished head was most frequent with respect to the effect on sperm head morphology.

(ii) Human system: The first study on the evaluation of genetic toxicity on 16 male insecticide applicators with variable and mixed exposures mainly to trichlorfon, malathion, diazinon, carbaryl, dicofol, endosulfan and DDT were done by Yoder et al. in 1973. The chromosomal preparations from blood samples showed a marked increase

in the mean chromatid break frequency. As no additional study on these insecticides had been done, no specific insecticide could be identified as mutagenic.

In another study Nazarethrabello et al. (1975) compared the workers of three insecticide plants in direct contact with DDT (2,2-bis (β -chlorophenyl)-1,1,1-trichloro ethane) to see the cytogenetic effects. The results were negative when the frequencies of chromosomal aberrations in the workers in direct contact with DDT were compared with control population of same plant who were not in direct contact. However, when the control group from one of the three plants which showed high DDT plasmic levels, was added to the group in direct contact with the insecticide, the frequency of cells with chromatid aberrations was significantly higher, suggesting that DDT causes chromatid lesions. Lessa et al. (1976) found no correlation between chromosomal aberrations and dose of DDT, in human leucocyte culture. However, at certain concentrations of DDT, the proportions of cells with structural chromosome aberrations was significantly greater than the control.

Kucerova et al. (1976) tested the mutagenic effects of epichlorhydrin on human lympocyte in vitro and compared with the mutagenic effects of TEPA. They found that the

effects of epichlorhydrin was five times lower than TEPA at same concentrations. Later Picciano, in 1978 confirmed the above results by reporting chromatid breaks, chromosome breaks, severely damaged and abnormal cells in the lympocyte culture of 93 workers exposed to epichlorhydrin.

Twentyfive workers engaged in venyl chloride and polyvenyl chloride production showed an increased frequency of sister chromatid exchanges in lympocyte cultures which increased with prolonged occupational exposure (Georgieva and Tsoneva, 1981).

II. CARBAMATE INSECTICIDES

Insecticidal properties of carbamates were known very recently eventhough they were used as weedicides since long. The carbamate insecticides like carbaryl, carbofuran, aldicarb, methomyl etc. are very commonly used today as systemic/nonsystemic insecticides for the control of stem borers, case worms, leaf rollers and bugs in rice; rhinoceros beetle, red palm weevil and coreid bugs in coconut; aphids, and thrips in cotton; stem borers in fruit and vegetable crops etc. Carbamate insecticides interfere with acetylcholinesterase enzyme involved in passing nerve impulses to muscle tissues, resulting in

paralysis and death of insects. The mutagenic properties of these group of insecticides are attributed to its reaction with nitrous acid to form N-nitroso methyl-naphthyl carbamate, a highly reactive compound.

(a) Plant system

The antimitotic properties of carbamate pesticides was reported by Morrison as early as 1962. He could observe inhibition of mitosis in plants treated with isopropyl-N-phenyl carbamate, ethyl carbamate, cytohexyl carbamate, and Avadax (2, 3 dichloroallyl diisopropyl thiocarbamate). Later in 1965, Amer found that in the roots of Allium cepa treated with Sevin (N-methyl-1-naphthyl carbamate) prepared from both pure and formulation at 22°C and 60°C for different periods of time, the end effect depended on the temperature of manufacturing in both pure and formulated Sevin. Solutions prepared at 22°C showed merokinetic tendencies, while that prepared at 60°C showed stathmokinetic tendencies and continuous treatment for twenty-four hours nearly arrested mitosis. Wuu and Grant (1967) observed strong inhibition of cell division by Sevin in barley (Hordeum vulgare). The colchicine mitotic activity of carbamates was reported by Storey et al. in 1968. They recommended it as a tool for class room

study of C-mitosis in plant cells. Amer and Farah (1968) while studying the effect of Sevin by spraying on flower buds of broad bean (Vicia faba) of different ages at different intervals (that is, two weeks old - spraying at weekly and fortnightly intervals for a month; one month old - spraying daily for a week and another daily spray for eight days) found that the percentage of abnormal pollen mother cells increased by increasing the number of sprays. Stickiness, lagging chromosomes and polyploid pollen mother cells were induced at different treatments. The haziness of chromosomes has been reported after treatment with chloropropham and propham by Herichova (1970).

Amer and Farah (1974a) investigated the effects of both pure and formulated (40 per cent active ingredient) Rogor (O, O-dimethyl-N-methyl carbamido-methyl-dithiophosphate) on mitosis of Vicia faba and Gossypium barbadense as seed soak and root treatments. It was found to affect mitotic index adversely in both the plants used. The percentage of abnormalities were more in formulated Rogor than in pure substance. Disturbed prophase, metaphase, and anaphase, and lagging chromosomes were common in both the plants. Chromosome contraction was noticed in G. barbadense while stickiness, fragmentation of chromosomes, anaphase bridges and multipolar anaphase were seen in V. faba.

Again in 1974(b), they compared the effects of two herbicides viz., IPC (O-isopropyl-N-phenyl-carbamate) and Duphar (a mixture of IPC + CIPC; CIPC is O-isoprophyl-N-(3-chloro) phenyl carbamate) with similar type of treatments on the same crops. Duphar was more effective in root mitosis of V. faba than G. barbadense roots. The anomalies were similar to the earlier studies except that multinucleated cells were also observed when treated with the latter chemicals. Further, with elaborate studies by Amer and Farah (1976) compared the secondary consequences such as meiosis, pollen viability, yield and root mitosis of carbamates like Rogor, IPC and Duphar with treatments like seed soak and spray at seedling and flowering stages on V.faba. Spraying with saturated solution of IPC, at flowering stage induced a relatively high percentage of abnormal pollen mother cells. All the three chemicals induced multipolar anaphase II and telophase II, in addition to stickiness, lagging and telophase bridges of chromosomes. IPC was capable of inducing tetraploid pollen mother cells also. The transmission of chromosomal aberrations to next generation was found to be very low. However, yield was significantly reduced in the first year of the treatment with 0.1 per cent Rogor and 0.5 per cent Duphar as seed treatment and also with 0.5 per cent Duphar

as spray at flowering stage. A significant increase in yield was found in second year, but the increase was diminished in the third generation.

Al-Najjar and Soliman (1980) reported high reduction in mitotic index, increase in the duration of metaphase stage and high percentage of chromosomal irregularities like anaphase bridges when seeds of Triticum aestivum, L., Triticum durum, Desf. and Aegelops linguistica were treated with Dithane S-60 and Vitavax-200 at the rate of 2 g/kg of seeds. Chromosome fragmentation was noticed in Dithane S-60 treatment apart from the above anomalies. In the meiotic studies on the effect of the same fungicides Soliman and Al-Najjar (1980) observed the production of abnormal pollen mother cells in wheat and two related species. Total abnormality of meiotic cells was more in A. linguistica, followed by T. aestivum and T. durum in the decreasing order. Asynchronization within the same cell in anaphase II was an interesting disorder, the other abnormalities being ring chromosomes, laggards and bridges at anaphase I and II. Laggards and bridges were the most frequent aberrations.

In a detailed study of pesticide genotoxicity in plant systems, Behera et al. (1982) screened four systemic

fungicides and a metabolite in Hordeum vulgare progeny test and observed that all the chemicals, viz., benomyl, carbendazin, thiophanate-methyl, dexon and dimethyl phenylene diamine affected germination, seedling growth, mitotic and meiotic activity, pollen fertility and seed set in M_1 generation to different degrees. The effects were much reduced in M_2 progeny. However, no chlorophyll mutation was induced in M_1 generation.

(c) Microbial system

Few reports of gene conversions and reverse mutations in microorganisms are available in the literature with carbamate pesticide treatments.

Parry (1973) conducted gene conversion studies in yeast (Saccharomyces cerevisiae) to test the mutagenicity of the herbicides viz., mecoprop, paraquot, gatnon, unden and their nitroso derivatives. Mecoprop, paraquot and dinoseb were found to induce gene conversion in yeast. Paraquot and dinoseb induced gene conversion at high survival levels whereas, mecoprap was active only at sublethal concentrations. Unden and gatnon displayed marked convertogenic activities. Highest frequency of mitotic gene conversion in yeast was reported by Siebert and Eisenbrand in the next year (1974).

The mutagenic activity of five methyl carbamate insecticide viz., carbaryl, baygon, buxten, landrin, and methomyl and their nitroso derivatives using histidine auxotrophs of Salmonella typhimurium derived by Ames was studied by Blevins (1977) and observed that nitroso derivatives alone could increase the number of revertant colonies. In 1980, Kar and Singh observed that the reversion frequency of a nonheterocystous nonnitrogen fixing (het^-nif^-) mutant of blue green algae (Nostoc muscorum) to heterocystous nitrogen fixing (het^+nif^+) to be 2.09×10^{-5} when treated with carbofuran which was almost equal to the mutagenicity (2.01×10^{-5}) of N-methyl-N-nitro-N-nitroso-guanidine (NTG), a known mutagen.

(c) Submammalian system

One of the reports available on the cytogenetic effects of carbamate insecticides is from Vasudev and Krishnamurthy (1981), who tested the toxic and mutagenic effects of baygon (2-isopropoxyphenyl-N-methyl carbamate) in Drosophila melanogaster by employing larval and adult feeding methods. There were no dominant lethal, sex linked recessive lethals or translocations upto 12.5 ppm in the larva. However, there were significant effect on viability of larvae in concentrations above 6.25 ppm.

They reported that baygon is neither clastogenic nor mutagenic as the highest concentrations of 1000 ppm tried was also unable to induce any significant sex linked recessive lethals or translocations in adult flies.

(d) Mammalian system

(i) Animal system: Cheng and Conner (1982) examined the *in vivo* sister chromatid exchange induction by venyl and allyl carbamates in alveolar macrophage, bone marrow and liver cells of C57BL/6J X DBA/2J F_1 mice. Both the chemicals indicated a striking similarity in relative potencies for sister chromatid exchange induction and their known bimorigenic potencies.

(ii) Human system: Ahmed *et al.* (1977) observed that the carbamate insecticides such as carbaryl irreversibly altered the human cellular DNA *in vivo* resulting in numerous alkali sensitive bonds. The nitroso derivative of carbamates like aldicarb, baygon, buxten, carbofurage, lendrin and methomyl induced numerous DNA breakage in human cells (Elevins *et al.*, 1977). The DNA repairing event could not occur after treatment of the chemicals as otherwise taking place in ultra voilet rays induced damages. The parent chemicals, however, did not show much breaks in DNA.

III. ORGANOPHOSPHATIC INSECTICIDES

The systemic and nonsystemic insecticides of organophosphate group is used to control effectively the sucking insects like fruit-flies, jassids, thrips, borer pests and other bugs of various crop plants. In rice, leaf folder, brown plant hopper, stem borer and whorl maggots are effectively controlled by monocrotophos and phorate. Banana aphids and leaf insects of vegetables are controlled by phorate and phosphamidon. Malathion is recommended for pests of vegetable and fruit crops. In coconut, red palm weevil and black headed caterpillar are controlled by dichlorvos and quinalphos respectively.

The toxicity of organophosphatic insecticides is mainly due to the blocking of cholinesterase enzyme. This occurs by the phosphorylation of the enzymes by the insecticides. The cytogenetic effects in various test systems as found by different authors are as follows.

(a) Plant system

The cytological effects of two organophosphorus systemic insecticides viz., Dimecron-100 and Rogor-40 with different concentrations were studied by Reddy and Rao (1969) on the broad bean plant. They noticed chromosome and chromatid breaks, dot deletions, fragments and anaphase

bridges in both metaphase and anaphase stages of mitosis. A maximum of 7.08 per cent aberrant cells were noticed in 0.1 per cent concentration of Rogor-40 as against 5.97 per cent in the same concentration of Dimecron-100. The same concentration produced 9.7 per cent and 4.7 per cent meiotic aberrant cells in Rogor-40 and Dimecron-100 respectively. A lower concentration of 0.05 per cent showed a mean of 7.18 per cent and 3.39 per cent aberrant cells treated with Rogor-40 and Dimecron-100 respectively.

Bladex and phosdrin when sprayed separately at 200 to 600 ppm on Vicia faba and Tradescantia, were found to be lethal to both the plants. The lethality was ascribed to be due to the increasing frequency of chromosomal abnormalities induced by the chemicals (Ahmed and Grant, 1972). The mitotic index in root meristem cells of Allium cepa was decreased when Mishra and Sinha (1979) gave treatments with different concentrations of malathion ranging from 64.73 to 4142.8 ppm. The chromosome aberrations like stickiness, fragmentation and laggards were observed. Amer and Farah (1979) studied the effects of leptophos (O,4-bromo-2, 5-dichlorophenyl O-methyl phenyl thio phosphonate) on seed germination and root mitosis of V. faba. Of the treatments given, seed soak for 48 and 72 hours showed marked inhibition of cell division and various

anomalies recorded were disturbed prophase, metaphase and anaphase. Lagging and fragmentation of chromosomes were also observed. Another insecticide phosvel (O-(4-bromo-2, 5-dichlorophenyl) O methyl phenyl thiophosphonate) when sprayed at two levels of concentrations at seedling and flowering stages of V. faba induced meiotic and chromosomal irregularities like disturbed second metaphase and anaphase, stickiness, laggards, fragments, anaphase bridges, univalents in diakinesis, micronuclei in the first and second telophase stages and multipolar telophase II (Amer and Farah, 1980). Phosvel did not show significant effect on pollen viability at the end of meiosis.

Panda and Sharma (1980) assessed the toxicity of trichlorfon and dichlorvos to the chromosomes of embryonic meristems and sporogeneous tissue by the progeny test of Hordeum vulgare. The effects on comparison with ethane methane sulfonate (EMS), a known mutagen were that dichlorvos surpassed the effects of EMS at concentrations of 1000 to 1500 ppm but trichlorofon was less effective than dichlorvos and ethane methane sulfanate but the effects were significant when tested. The frequency of late effects in the pollen mother cells was higher than that of initial effects in the embryo shoot cells and this differential cellular response was thought to be due to the cryptic nature of aberrations in embryo shoot cells.

(b) Microbial system

In agar plate test with Escherichia coli, WP_2 Smith et al. (1972) reported that dimethoate (dimethyl-S-N-methyl carbamyl methyl phosphoro thiolo thionate) and bidrin appeared to be nonmutagenic. The DNA strand breakage in E. coli caused by the effect of dichlorvos and methyl methane sulfonate was found to be repaired by DNA polymerase enzyme (Bridges et al., 1973). Of the eight organophosphate insecticides tried by Mohn (1973) in E.coli, three viz., dimethoate, bidrin and oxydemeton methyl were found to be mutagenic. Methyl parathion exhibited low toxicity in this system. Fahrig (1974) tested the mutagenic activity of several mono-and di-alkyl esters of phosphoric acid and thiophosphoric acid, products of the partial hydrolysis of organophosphate insecticides in vitro in E. coli and yeast (Saccharomyces cerevisiae). He found that para-Nitrophenol, a metabolite of parathion, and methyl parathion were not genetically active in E. coli, but induced gene conversion in yeast. As many as 140 organophosphorus compounds were tested for their mutagenicity in bacteria by Hanna and Dyer (1975). Out of these, 28 compounds were found to be mutagenic.

Shirasu et al. (1976) surveyed the mutation induction capacity of 166 pesticides using rec assay procedure

utilising H_{17} Rec+ and M_{45} Rec⁻ strains of Bacillus subtilis, Escherichia coli WP2 and Salmonella typhimurium. Out of the pesticides screened, captafol, captan, dexon, dichlorvos, NBT (2,4-dinitrophenyl thiocyanate), folpet and NNN (5-nitro-1-naphthonitrite) were found to be mutagenic in these tests. In another study, Darell et al. (1978) assessed the in vitro breakage of plasmid DNA by mutagens and pesticides. Of the 11 pesticides tested, positive results were obtained for dexon, DDVP, malathion and methyl parathion. They induced breaks in the plasmid molecules at a significantly higher rate than controls.

Srivastava and Sarma (1979) tested the effects of dimecron and nuvan on the cytology, survival and growth of fungus, Didymonium gunnii. The fragmentation of chromosomes was occasionally seen with 250, 500 and 800 ppm. Asynchronous cellular and nuclear divisions were also observed The concentrations of 300 ppm dimecron and 250 ppm nuvan were lethal for the zoospores. The lethal concentrations for the mature filaments was 1000 ppm after 48 hours and 72 hours of dimecron and nuvan. The lowest percentage of survival of zoospores was 8.1 per cent with 250 ppm of dimecron and 8.00 per cent with 100 ppm of nuvan.

(c) Submammalian system

Injection of subtle amounts of bromophos, fenitrothion and trichlorophon in Drosophila melanogaster did not increase the frequency of dominant lethal mutations, even when 80 ppm dose was given (Benes et al., 1973; Wild, 1975). When Hanna and Dyer (1975) exposed seven organophosphorus insecticides for 18 months on D. melanogaster found that six of them were capable of inducing recessive lethal mutations. Dichlorvos did not have any mutagenic effect in D. melanogaster (Kramers and Knaap, 1978; Sobels and Todd, 1979). Their individual reports showed that adults and larvae did not give positive results when they were fed with the chemical in the medium.

Dhingra and Vijayakumar (1981) reported that dimethoate and malathion were not genotoxic in D. melanogaster from their experiment by administering different concentrations of insecticides to eggs, larva (III instar) and adult flies. The adults were most susceptible and they concluded that mutagenicity of the insecticides was over shadowed by physiological toxicity.

(d) Mammalian system

(i) Animal system: Dean and Thorpe (1972) found that the frequency of cells with chromatid aberrations

including gaps ranged between 0.17 and 1.25 per cent in dichlorvos treated mice bone marrow and testis cells, which were not significantly different from spontaneous frequencies. Epstein (1972) confirmed the above results by both intraperitonial injection and oral feeding at 13 to 15.5 mg per kg and 5 to 10 mg per kg body weight respectively. In pigs parathion induced significantly higher frequency of chromosome fragments ranging from 11.5 to 18.5 per cent by intratesticular administration (Dikstith, 1973).

Repeated oral doses of 1 mg per kg of body weight, each of malathion and parathion over a period of seven days induced chromosomal changes like fragments, stickiness, C-mitosis, gaps etc. in rats (Giri et al., 1978). Degraeve et al. (1979) reported that injections of malathion upto 300 mg per kg and of dimethoate and dichlorvos at 10 mg per kg each to mouse showed no cytogenitic effects and dominant lethal mutations. Bhojvaid (1980) studied the effects of malathion in swissalbino mice and found no significant increase in the frequency of chromosomal abnormalities when compared to the controls.

Out of the eight organophosphate insecticides tried by Chen et al. (1981) on Chinese hamster V 79 cells, six were found to induce significant increase in the

frequency of sister chromatid exchange while diazin and disyston had no effect in sister chromatid exchange. Methyl parathion, dimeton, trichlorfon, dimothoate, malathion and methidathion showed delay in cell cycle. They recommended that the cell cycle delay is a sensitive mechanism for assessing the mutagenicity of environmental pollutants.

Giri et al. (1981) when analysed liver lung and kidney after administering malathion and parathion at the rate of 1 mg per kg body weight of albino rats over a period of seven days, found that DNA and RNA contents were increased in liver, kidney and lung cells, but protein increased in lungs.

(ii) Human system: The chromosome analysis of 31 persons intoxicated by organophosphorous acid esters showed a significant increase in abnormalities, thereby indicating probable mutagenic effects of insecticides containing this compound (Cseizel et al. 1973). In another investigation, the chromosomes of 42 pesticide applicators and 16 controls were analysed by Yoder et al. (1973). A marked increase in chromatid lesions were observed. The insecticides most commonly used by these exposed individuals included parathion, malathion, dicofol, endosulfan, methyl parathion, dimethoate, DDT and carbaryl.

VanBao *et al.* (1974) collected blood samples from the patients intoxicated with methyl paration (5 patients), malathion (14 patients), trichlorfon (5 patients) a few by dimethoate, dichlorvos and diazinon, at different intervals. The samples collected immediately after the intoxication showed eight fold increase in chromatid breaks compared with healthy control. After six months of intoxication, the abnormal frequencies approached control indicating nonresidual effect of the insecticides.

Georgian (1975) compared the cytogenetic effects of aldrin and phosphamidon, when these were administered in the human lympocyte cultures. Cultures treated with phosphamidon showed a high frequency of aberrant cells and chromosomal abnormalities in comparison to the ones treated with aldrin. In a cytogenetic investigation of workers engaged in the manufacture of organophosphorus insecticides the frequency of chromatid-type of aberrations was found to be higher than that of control population (Kiraly *et al.*, 1977).

Nicholas *et al.* (1979) in a study of sister chromatid exchange in human foetal fibroblast culture by exposure of malathion found significant increase at 40 g per ml and a double exposure at 20 g per ml at 20 hours gap showed a cumulative effect.

Sobti et al.(1982) found that cytotoxic effects were dose related and often lead to cell-death. In their investigation of cytotoxic, cytostatic and cytogenetic effects of a number of organophosphate insecticides on human lympoid cells (LAZ-007) in culture, 11 out of 14 insecticides tested significantly increased sister chromatid exchange frequency. Diazinon, dimethoate, dursban and phosdrin treated liver microsomal S-9 preparations showed significant increase in sister chromatid exchange.

Materials and Methods

MATERIALS AND METHODS

The present investigation was undertaken in the Division of Agricultural Botany, College of Horticulture, Vellanikkara, during the year 1981-83.

MATERIALS

A. The Test System

Common onion (Allium cepa, L.) was used as the test system in this study. Onion is a classic test system for assaying chromosomal aberrations ever since 1938, when it was used for the first time by Levan for studying chemical effects on chromosomes. This species is advantageous for assessing the cytogenetic effects of environmental chemicals, because it is simple, reliable and inexpensive to carry out the necessary experimentation. Onion bulbs are available year round and they produce large number of roots in a short time. The chromosomes are relatively large averaging from 8 to 16μm in length (Grant, 1982b), depending on the stage of division. Hence the aberrations can be detected easily. It has also been reported to be much more sensitive than broad beans (Vicia faba, L.) to the chemical effects on chromosome structure (Khilman, 1966). The karyotype of Allium cepa has been described by Mensinkai (1939). The complement

consists of eight pairs of chromosomes (2n = 16) as follows: 5 pairs of chromosomes with centromere situated median to submedian (arm ratios: 1.02, 1.05, 1.1, 1.3 and 1.4; chromosomes: 1, 2, 3, 5 and 8); two pairs in which the centromeres are submedian (arm ratios: 1.6 and 1.8; chromosomes: 6 and 7); and one pair of satellite chromosomes in which the satellites are situated at the end of the short arm (arm ratio: 3.4; chromosome:4).

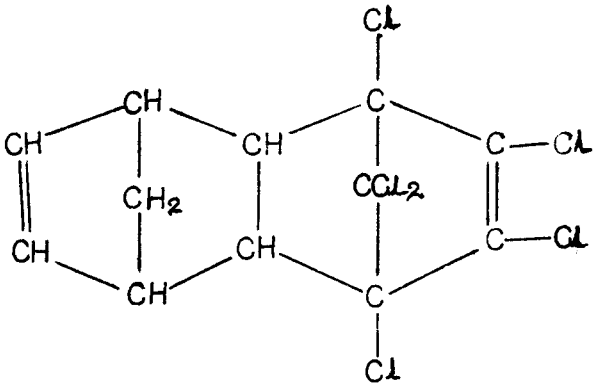
Gonsalez-Fernandez et al. (1966) in a study have estimated that the division cycle of root tip cells is 13.5 hours. In this division cycle, interphase lasts for 11.2 hours and mitosis 2.3 hours, divided into prophase of 64 minutes, metaphase of 18 minutes, anaphase of 13 minutes and telophase of 42 minutes durations.

B. Insecticides

Three very commonly used insecticides viz., aldrin (as Aldrex containing 30 per cent aldrin), carbofuran (as pure technical material) and phorate (as Thimet containing 10 per cent phorate) were selected for this study. While two of these chemicals, carbofuran and phorate are systemic in their action, aldrin is a nonsystemic broad spectrum insecticide. All these insecticides are widely used in agriculture to protect the crops from various soil borne as well as direct crop insects and therefore the chances of

their getting in contact with nontarget organisms are very much. These three insecticides represent the three major groups of pesticides, namely, chlorinated hydrocarbons, carbamates and organophosphates in the order aldrin, carbofuran and phorate. A brief description of these chemicals as given by Worthing (1979) is furnished below.

1. Aldrin

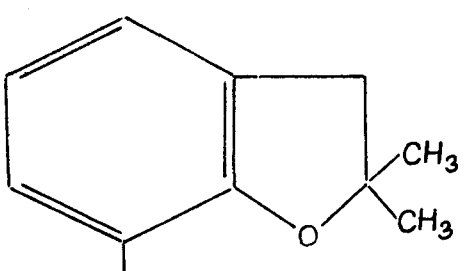
Common name	:	Aldrin, HHDN
Trade name	:	Aldrex
Chemical name	:	1,2,3,4,10,10-hexachloro-1α, 4α, 4aβ, 5α, 8α, 8aβ hexahydro-1, 4:5, 8-dimethano naphthalene
CA number	:	309-00-2
Molecular formula	:	$C_{12}H_8Cl_6$
Molecular weight	:	364.9
Structural formula	:	
Introduced by	:	J.Hyman, and company as compound 118, under the trade mark Octalene in 1948. Its insecticidal property was described in 1949.

Chemistry	: It is a colourless crystalline solid, melting point (M.P.) 104 to 104.5°C, vapour presure (V.P.) 7.5×10^{-5} mm Hg at 20°C, 1.4×10^{-4} mm Hg at 25°C. The technical grade is tan to dark brown solid with M.P. 49 to 60°C containing 85 per cent HHDN.
Solubility	: 27µg/1. water at 27°C, moderately soluble in petroleum oils, readily soluble in acetone, bensene and xylene.
Stability	: Aldrin is stable to heat, to alkaline and dilute acids, but oxidising agents and concentrated acids attack the unchlorinated ring. It is compatible with most pesticides and fertilizers but corrosive because of the slow formation of hydrogen chloride on storage.
Uses	: Aldrin is a nonsystemic and persistant insecticide, effective against soil insects at rates of 0.5 to 5.0kg/ha and is non-phytotoxic. It is readily oxidised to dieldrin.

Formulations	: These include : e.c.(240-480 g/l), W.P.(400-700 g/kg), urea may be added to prevent dehydrochlorination by certain carriers; dusts (25-50 g/kg); seed dressings; granules.
Toxicology	: LD_{50} rat oral: 67 mg/kg body weight. It is absorbed through skin also. In two year feeding trials rats receiving 5 mg/kg body weight suffered no ill-effect, but liver changes resulted at 25 mg/kg diet.
Source for the present study	: M/s. National Organic Chemical Industries Ltd., Bombay, as Aldrex 30 EC.

2. Carbofuran

Common name	: Carbofuran
Synonyms	: ENT 27164, Furadan, Curaterr, BAY-70413, FMC 10242.
Chemical name	: 2,3-dihydro-2, 2-dimethyl-7-benzofuranyl methyl carbamate.
CA number	: 1563-66-2.
Molecular formula	: $C_{12}H_{15}NO_3$
Molecular weight	: 221.3
Structural formula	:



Introduced by	: The Agricultural chemical division of the FMC Corporation in 1967.
Chemistry	: Carbofuran is a colourless crystalline solid, melting point 150 to 152°C; vapour pressure 2 x 10^{-5} mm Hg at 33°C d_{20}^{20} 1.80.
Solubility	: 700 mg/l water at 25°C; 150 g/kg acetone; 40 g/kg benzene.
Stability	: It is unstable in alkaline media, noncorrosive and noninflammable. It is metabolished in the liver and excreted in the urine of animals 50 per cent being lost in 6 to 12 hours; in soil 50 per cent is lost in 30 to 60 days. 2,3-dihydro-3-hydroxy-2, 2-dimethyl benzofuran-7-yl methyl carbamate, which is low toxic to insects and nematodes, is one of the products formed.
Uses	: It is a systemic insecticide, acaricide and nematicide applied to foliage at 0.25 to 1.0 kg ai/ha for the control of insects and mites or applied to the seed furrow at 0.5 to 4.0 kg/ha to control soil pests and foliar feeding insects or broadcast at 6 to 10 kg/ha for the control of nematodes.

Formulations : This include W.P. (750 g ai./kg), flowable paste (480 g/kg); granuales (20, 30, 50 and 100 g/kg).

Toxicology : LD_{50} rats-acute oral: 8-14 mg ai. (in corn oil)/kg body weight. LD_{50} dogs-acute oral: 19 mg ai. dry powder/kg body weight. LD_{50} rabbits dermal: 2550 mg ai. as W.P./kg body weight.

Source for the present study : Rallis India Ltd., Bombay, as 100 per cent technical ingredient of carbofuran.

3. Phorate

Common name : Phorate

Synonyms : Thimet, EI-3911, ENT 24042.

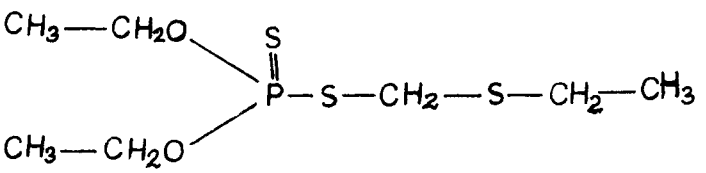
Chemical name : 0,0-diethyl S-ethylthiomethyl phosphorodithioate.

CA number : 289-02-2.

Molecular formula : $C_7H_{17}O_2PS_3$

Molecular weight : 260.4

Structural formula:



Chemistry : It is a clear mobile liquid, boiling point (B.P.)118-112°C per 0.8 mm Hg. The technical grade d^{25}-1.167 is >90 per cent pure.

Solubility : Soluble in water at the rate of 50 mg/l at room temperature. It is miscible with carbon tetrachlorodioxane, vegetable oils, xylene, alcohols, ethers and esters.

Stability : It is stable at room temperature. Environmental stability optimum in the range pH 5 to 7. Highly acidic (pH > 2) or alkaline (pH>9) media promote hydrolytic decomposition at rates depending on temperature and pH. In plants and animals it is metabolically oxidised at both the thioether linkage and co-ordinate sulphur yielding sulphoxide and sulphone and their phosphothioate analogues as both the parent and oxidation products are readily hydrolysed, only a small portion of the sulphone results.

Uses : Systemic and contact insecticide and acaricide used to protect crops primarily root and field crops like cotton,

	brassica, coffee, rice etc. from sucking and biting insects, mites and certain nematodes. Soil insecticide for maize and sugar beet.
Formulations	: These include e. c. of various active ingredients-Thimet LC-8 (960 g tech/l) also (200 and 250 g ai./l) and granules (10, 50, 100, 150 g/kg).
Toxicology	: LD_{50} rat-oral: 1.6 to 3.7 mg/kg body weight; dermal: 2.5 to 6.2 mg/kg body weight.
Source for the present study	:Cyanamid India Ltd., Bombay, as Thimet 10G (containing 10 per cent active ingredient).

C. Fixative

Carnoy's fluid was used for fixing onion roots after treatment. This was prepared by mixing one part of glacial acetic acid and three part of absolute ethyl alcohol.

D. Stain

Feulgen stain 0.5 per cent was used for the mitotic studies in the present investigation. The stain was prepared by the method given by Darlington and LaCour (1976).

One gramme basic fuchsin was dissolved in 200 ml boiling distilled water. The solution was cooled to 50°C and filtered through Whatman No.1 filter paper. Thirty ml of 1N HCl and 3 g potassium metabisulphate ($K_2S_2O_5$) were added to the filtrate. It was kept in dark for 24 hours in a tightly stoppered bottle. To this solution was added 0.5 g of activated charcoal, shaken well and filtered through Whatman No.1 filter paper. The colourless stain obtained was stored in a well stoppered bottle covered with black paper at 4°C.

METHODS

A. Treatments

1. Germination of onion bulbs

The young and healthy bulbs of common onion (*Allium cepa*, L.) of relatively uniform size (about 10 to 25 g in weight) and which would root profusely, were selected for the study. The bulbs were denuded by removing the loose outer brown scales. The base of the bulbs were scrapped gently and carefully to remove the old and dried roots and then to expose the apices of the root primodia. The bulbs were then sown in a pure sand tray moistioned with tap water for two days for germination of roots under laboratory conditions.

2. Fixing the doses of insecticides

The concentrations for the treatments were fixed taking into consideration the recommended dosage for field application in the control of pests. Three concentration, viz., the concentration of normal field application, one dose above and one below were selected for each chemical. Wherever the lower concentration selected was capable of inducing considerable degree of chromosomal aberrations, another lower dose was also selected. The different concentrations selected for each insecticide are given below.

(i) Aldrin	: 0.03 per cent, 0.05 per cent, 0.10 per cent and 0.15 per cent.
(ii)Carbofuran	: 0.0075 per cent, 0.01 per cent, 0.02 per cent and 0.04 per cent.
(iii)Phorate	: 0.02 per cent, 0.03 per cent 0.06 per cent and 0.09 per cent.

3. Giving the treatments

While the stock solution of phorate and carbofuran were made in 2 ml acetone and the volume further made up by distilled water, aldrin was dissolved in distilled water alone.

For giving the treatments with different concentrations of the insecticides, onion roots grown for 48 hours

were used. These bulbs with roots intact were washed thoroughly in running water and transferred to the vials containing freshly prepared treatment solutions of the insecticides of various concentrations as given above. Care was taken to immerse all the growing roots in the treatment solution. The treatments were stopped after a particular time interval by lifting the onion bulbs from the vials and washing them thoroughly in running water. All the treatments were given for three treatment periods viz., 12, 24 and 48 hours.

Separate control experiments were also carried out with the three time intervals (12, 24 and 48 hours) in distilled water as well as in 0.2 per cent acetone.

For each treatment as well as control, ten germinated onion bulbs were used.

B. Mitotic studies

1. Fixation

The roots of onion bulbs were washed thoroughly after treatment period was attained and then the roots were excised and fixed in 1:3 acetic-alcohol for 24 hours. The fixed roots were stored in 70 per cent alcohol in a refrigerator, whenever the mitotic studies have to be done in subsequent days.

2. Staining

The roots were thoroughly washed in distilled water to remove excess fixative or ethanol as the case may be. They were then hydrolysed in 1N HCl at 60°C for 6 to 7 minutes in a waterbath. These roots were stained in Feulgen stain for 5 to 10 minutes, after washing out the hydrochloric acid from them.

3. Slide preparation

The meristematic region of the roots which had attained the characteristic magenta colour were put on the slide after removing the rest of the root portion. A drop of water was added and the root tip was covered with a coverslip and tapped gently. Better spreads were obtained by pressing the coverslip after keeping the slide in filter paper folds. The slides were sealed with clear finger nail polish to provide a temporary mount for mitotic studies under microscope.

The slides were made permanent after removing the coverglass by dipping them inverted in 45 per cent acetic acid. The slides were then passed through acetic acid and butanol series in the proportions 3:1, 1:1, and 1:3. Finally the slides were dehydrated in butanol alone and mounted in canada balsam.

The photomicrographs of typical abnormalities were taken in a Lietz 3 mm photomicroscope using blue filter in ORWO NP22 slow negative film at 322.56 × magnification.

4. Mitotic index

Two to three random fields from each slides were scanned for scoring dividing and nondividing cells in all the treatments and controls. The dividing cells included those showing any stages of cell division, such as prophase, metaphase, anaphase and telophase. Mitotic index was calculated using the formula,

$$\text{Mitotic index (MI)} = \frac{\text{Number of dividing cells}}{\text{Total number of cells scored}} \times 100$$

About 4000 cells from 10 to 15 slides prepared from each of the treatments were scanned for this. Indices of different division stages were also calculated by the following formulae.

$$\text{Prophase index (Pi)} = \frac{\text{Number of prophase cells}}{\text{Total number of dividing cells}} \times 100$$

$$\text{Metaphase index (Mi)} = \frac{\text{Number of metaphase cells}}{\text{Total number of dividing cells}} \times 100$$

$$\text{Anaphase index (Ai)} = \frac{\text{Number of anaphase cells}}{\text{Total number of dividing cells}} \times 100$$

$$\text{Telophase index (Ti)} = \frac{\text{Number of telophase cells}}{\text{Total number of dividing cells}} \times 100$$

5. Relative cell division rate (RDR)

In order to have a comparative estimate of the effect of different treatments on mitosis with respect to controls, the relative division rate was computed. The relative cell division rate in the treated variants was computed using the modified equation of Egami and Hyado-Taguchi (1973) as cited by Mishra and Sinha (1981).

$$\text{Relative cell division rate (RDR)} = \frac{\text{Per cent of cells in division in treated} - \text{Per cent of cells in division in control}}{\text{100-per cent of cells in division in control}} \times 100$$

The relative cell division rate of total division and in different mitotic division phases were also calculated.

6. Scoring of chromosomal abnormalities

The slides prepared from the root tips of the treated and control experiments were scanned throughly for various types of abnormalities in different stages of cell division. About 100 to 150 cells in each stage of division (prophase, metaphase, anaphase and telophase) in each treatments were scanned. The aberrations which were scored included stickiness, breaks, nonorientation of chromosome at the equatorial plate of metaphase, C-metaphase, star metaphase, laggards, chromosome bridges, micronuclei, chromatin bridges etc.

The total frequency and percentage of abnormality in each division stage were computed. Chi-square (χ^2) test was used to compare the effect of each treatment against control with respect to the aberrations induced. The Chi-square test was done using the following formula.

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

The significance of variances of different treatments with the control was tested using the χ^2 table at five per cent and one per cent levels.

RESULTS

Results of various cytological observations made in *Allium cepa*, L., root meristems treated with three most commonly used insecticides viz., aldrin, carbofuran and phorate, in order to assess their cytotoxicity and clastogenicity along with proper control treatments are presented below. The visible anomalies, mitotoxicity in general, and chromosomal aberrations in particular under microscope were used as the parameters for this study. Likewise, the results are presented in two separate subheads, as cytotoxicity and chromosomal abnormalities.

A. CYTOTOXICITY

The cytotoxicity of the selected insecticides were determined based on the mitotic indices in well spread fields on the slides prepared from treated root tip cells in comparison with control experiments. The effects of each insecticide used are given separately hereunder.

In the control experiment using distilled water for 12 hours, the mitotic index recorded was 7.70. It was reduced to 7.40 and 6.52 when the time of treatments were increased to 24 and 48 hours respectively. The indices of different stages of cell division when analysed, it was

found that prophase index was 51.65, metaphase index 11.54, anaphase index 9.89, and telophase index 26.92 in 12 hours of treatment. At 24 hours the spread spectrum of mitotic index were 56.44, 11.04, 4.60 and 27.91 for prophase, metaphase, anaphase and telophase respectively. The values were 55.41, 9.84, 6.23, and 28.52 respectively for the different division phases at 48 hours in distilled water. In each period 4728, 4406 and 4679 cells were examined for the computation of these indices.

In another control experiment, 0.2 per cent acetone was used since this was the solvent for two of the pesticides used viz., carbofuran and phorate. The mitotic indices for 12, 24 and 48 hours of treatments were 5.62, 5.58 and 4.08 respectively. Prophase, metaphase, anaphase and telophase indices were 37.63, 23.20, 13.92 and 25.26 respectively at 12 hours and 36.32, 18.91, 16.42 and 28.36 at 24 hours. At 48 hours these indices were 42.21 for prophase, 13.64 for metaphase, 9.74 for anaphase, and 34.42 for telophase.

1. Aldrin

Table 1 gives the mitotic index and the indices of different stages of cell division with varying concentrations of aldrin treatment. Aldrin in general reduced the mitotic index in Allium cepa. The mitotic index was 3.64

Table 1. Cytotoxic effects of different concentrations of aldrin in *Allium cepa*, L.

Concentration (percentage)	Treatment period (hours)	Total number of cells examined	No.of dividing cells	Mitotic index	Stages of division							
					Prophase		Metaphase		Anaphase		Telophase	
					Total	Index	Total	Index	Total	Index	Total	Index
0.03	12	3657	133	3.64	54	40.60	29	21.80	14	10.53	36	27.07
	24	2226	101	4.54	46	45.54	22	21.78	7	6.93	26	25.74
	48	3161	143	4.52	47	32.87	26	18.18	21	14.69	49	34.27
Mean of periods	treatment	-	-	4.23	-	39.67	-	20.59	-	10.72	-	29.03
0.05	12	3217	99	3.08	39	39.39	25	25.25	11	11.11	24	24.24
	24	3267	117	3.58	39	33.33	27	23.08	9	7.69	42	35.90
	48	3180	74	2.33	21	28.38	12	16.22	10	13.51	31	41.89
Mean of periods	treatment	-	-	3.00	-	33.70	-	21.52	-	10.77	-	34.01
0.10	12	3078	67	2.18	26	38.81	10	14.93	11	16.42	20	29.85
	24	3208	78	2.43	30	38.46	10	12.82	6	7.69	32	41.03
	48	3460	35	1.01	12	34.29	6	17.14	4	11.43	13	37.14
Mean of periods	treatment	-	-	1.87	-	37.19	-	14.96	-	11.85	-	36.01
0.15	12	2836	74	2.61	30	40.54	16	21.62	9	12.16	19	25.68
	24	2725	64	2.35	9	14.06	6	9.38	3	4.69	46	71.88
	48	2740	46	1.68	16	34.78	10	21.74	6	13.04	20	43.48
Mean of periods	treatment	-	-	2.21	-	29.79	-	17.58	-	9.96	-	47.01
Control (distilled water)	12	4728	364	7.70	188	51.65	42	11.54	36	9.89	98	26.92
	24	4406	326	7.40	184	56.44	36	11.04	15	4.60	91	27.91
	48	4679	305	6.52	169	55.41	30	9.84	19	6.23	87	28.52
Mean of periods	treatment	-	-	7.21	-	54.50	-	10.81	-	6.91	-	27.78

at 12 hours with 0.03 per cent of aldrin and the increase of treatment period from 12 hours to 24 hours increased the mitotic index to 4.54. However, further increase of period to 48 hours did not bring about any notable change as the decrease was only from 4.54 to 4.52 (difference of 0.02) when 3161 cells were observed. The spread spectrum of indices in different division stages showed an accumulation of metaphase and anaphase cells compared to the control and decrease in prophase indices. The telophase indices of control and 0.03 per cent aldrin treated cells did not vary considerably.

The 0.05 per cent concentration of aldrin reduced the mitotic index to 3.08, 3.58 and 2.33 at 12, 24 and 48 hours respectively. These were reflected as decreased prophase index and increased metaphase and anaphase indices.

The normal dosage of field application (0.10%) showed much more inhibition in cell division with mitotic indices 2.18, 2.43 and 1.01 at 12, 24, and 48 hours respectively. The anaphase and telophase indices were found to be increasing in this dosage.

The highest dosage tried was 0.15 per cent and the mitotic indices were 2.61, 2.35 and 1.68 at 12, 24 and 48 hours. The prophase and metaphase indices in 12 and 48 hours were found to be improving compared to the lower

FIG. 1 DOSE RESPONSE OF ALDRIN FOR MITOTIC INDEX

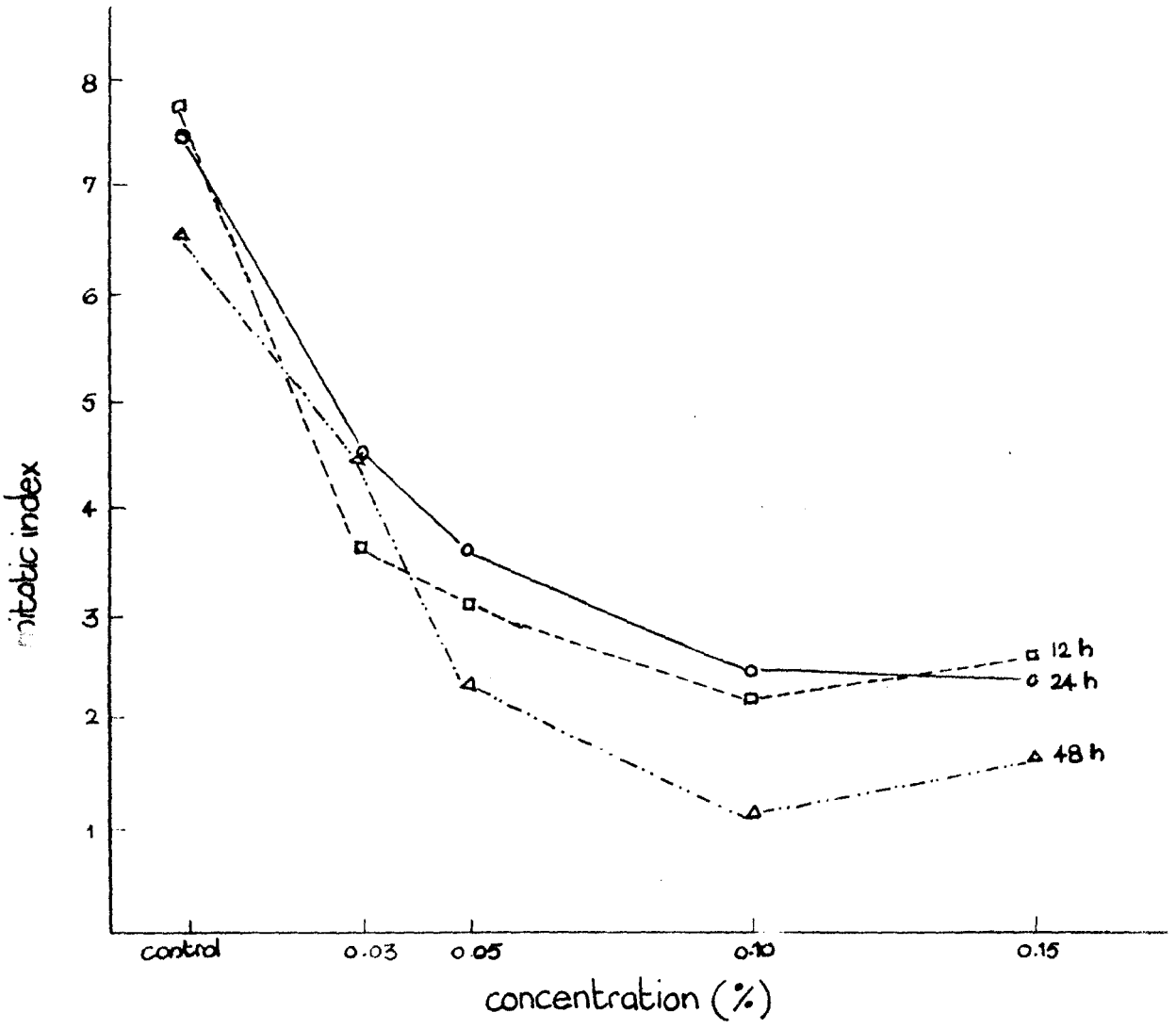


Table 2. Relative division rate of cells and mitotic phases in *Allium cepa*, L. treated with aldrin.

Concentration (percentage)	Treatment period (hours)	Percentage of dividing cells	Relative cell division rate (RDR)	Stages of division							
				Prophase		Metaphase		Anaphase		Telophase	
				Percentage	RDR	Percentage	RDR	Percentage	RDR	Percentage	RDR
0.03	12	3.64	-4.40	1.48	-2.60	0.79	-0.10	0.38	-0.38	0.98	-1.11
	24	4.54	-3.09	2.07	-2.20	0.99	+0.17	0.31	-0.03	1.17	-0.91
	48	4.52	-2.14	1.49	-2.58	0.82	+0.18	0.66	+0.25	1.55	-0.32
0.05	12	3.08	-5.01	1.21	-2.88	0.78	-0.11	0.34	-0.42	0.75	-1.35
	24	3.56	-4.15	1.19	-3.12	0.83	+0.01	0.28	-0.06	1.29	-0.79
	48	2.33	-4.48	0.66	-3.06	0.38	-0.26	0.31	-0.10	0.97	-0.91
0.10	12	2.18	-5.98	0.84	-3.27	0.32	-0.58	0.36	-0.40	0.65	-1.45
	24	2.43	-5.37	0.94	-3.38	0.31	-0.51	0.19	-0.15	1.00	-1.08
	48	1.01	-5.89	0.35	-3.38	0.17	-0.47	0.12	-0.29	0.38	-1.51
0.15	12	2.61	-5.51	1.06	-3.04	0.56	-0.33	0.32	-0.44	0.67	-1.43
	24	2.35	-5.45	0.33	-4.02	0.22	-0.60	0.11	-0.23	1.69	-0.38
	48	1.68	-5.18	0.58	-3.14	0.36	-0.28	0.22	-0.19	0.73	-1.15
Control (distilled water)	12	7.70	-	3.98	-	0.89	-	0.76	-	2.07	-
	24	7.40	-	4.18	-	0.82	-	0.34	-	2.06	-
	48	6.52	-	3.61	-	0.64	-	0.41	-	1.86	-

dosage of 0.01 per cent aldrin. The anaphase and telophase indices remain same in12 hours of treatment as that of the next lower dose (0.01%), while they were improved in the 48 hours of treatment. In 24 hours, the prophase, metaphase and anaphase indices decreased while telophase index increased when compared to 0.01 per cent treatment.

The dose response curve of mitotic index with aldrin treatment is presented in Fig.1. In order to have a comparative evaluation of the inhibition of cell division with respect to control the relative cell division rates are given in Table 2.

2. Carbofuran

The response spectrum of mitotoxicity with different concentrations of carbofuran is given in Table 3. At the lowest concentration used, namely, 0.0075 per cent, the mitotic index was 5.43 at 12 hours of treatment, while the other periods, 24 and 48 hours, decreased the value to 4.82 and 4.53. However, the mitotic index of 4.53 at 48 hours showed an increase in rate of division (+0.47) with respect to control. This can be seen from Table 4, which gives the relative cell division rates with carbofuran treatment. This increase of mitotic index was manifested

Table 3. Cytotoxic effects of different concentrations of carbofuran in Allium cepa, L.

Concentration (percentage)	Treatment period (hours)	Total number of cells examined	No.of dividing cells	Mitotic index	Stages of division							
					Prophase		Metaphase		Anaphase		Telophase	
					Total	Index	Total	Index	Total	Index	Total	Index
0.0075	12	3758	204	5.43	91	44.61	48	23.53	20	9.80	45	22.06
	24	3030	146	4.82	63	43.15	24	16.44	18	12.33	41	28.08
	48	3161	143	4.53	47	32.87	26	18.18	21	14.69	49	34.27
Mean of periods	treatment	-	-	4.93	-	40.21	-	19.38	-	12.27	-	28.14
0.01	12	3351	152	4.54	71	46.71	27	17.76	15	9.87	39	25.66
	24	3518	168	4.78	78	46.45	26	15.48	20	11.90	44	26.19
	48	3685	131	3.55	62	47.33	14	11.38	17	13.82	40	32.52
Mean of periods	treatment	-	-	4.29	-	48.82	-	14.87	-	11.86	-	28.12
0.02	12	3517	133	3.78	48	36.09	22	16.54	22	16.54	41	30.83
	24	3225	198	6.14	101	51.01	33	16.67	23	11.61	41	20.71
	48	3163	175	5.53	84	48.00	24	13.71	25	14.29	42	24.00
Mean of periods	treatment	-	-	5.15	-	45.03	-	15.64	-	14.15	-	25.18
0.04	12	3427	164	4.79	87	53.05	23	14.02	20	12.20	34	20.73
	24	3048	67	2.20	22	32.84	15	22.39	10	14.93	20	29.85
	48	2490	48	1.93	25	52.08	4	8.33	5	10.42	14	29.17
Mean of periods	treatment	-	-	2.97	-	45.99	-	14.91	-	15.52	-	26.58
Solvent control (acetone)	12	3455	194	5.62	73	37.63	45	23.20	27	13.92	49	25.26
	24	3607	201	5.58	73	36.32	38	18.91	33	16.42	57	28.36
	48	3771	154	4.08	65	42.21	21	13.64	15	9.74	53	34.42
Mean of periods	treatment	-	-	5.09	-	38.72	-	18.58	-	13.36	-	29.35

in the form of increase in metaphase and anaphase indices, while the other two treatment periods were capable to affect the prophase index.

Carbofuran at 0.01 per cent was inhibitive of cell division as expressed by the reduced mitotic index, that is, 4.54, 4.78, and 3.55 at 12, 24 and 48 hours of treatment respectively. While three treatment durations have increased the prophase index in comparison to the control, the metaphase, anaphase and telophase indices have recorded a slight reduction.

With 0.02 per cent carbofuran treatment for 12 hours, the mitotic index was reduced to 3.78 and with 24 and 48 hours treatments the values were 6.14 and 5.53 respectively. The 48 hours treatment increased the relative cell division rate values in all the phases except telophase.

Drastic mitodepression with a mitotic index value of 1.93 in comparison to 4.08 of the control was the observation made with 0.04 per cent of carbofuran treatment for 48 hours. With 12 and 24 hours the mitotic index values were 4.79 and 2.20. All the division phase indices also decreased except prophase index in 12 hours, which showed an increase of 0.44 from the control. The

Table 4. Relative division rate of cells and mitotic phases in *Allium cepa*, L. treated with carbofuran

Concentration (percentage)	Treatment period (hours)	Percentage of dividing cells	Relative cell division rate (RDR)	Stages of division							
				Prophase		Metaphase		Anaphase		Telophase	
				Percentage	RDR	Percentage	RDR	Percentage	RDR	Percentage	RDR
0.0075	12	5.43	-0.20	2.42	+0.32	1.28	-0.02	0.53	-0.25	1.20	-0.22
	24	4.82	-0.74	2.08	+0.06	0.79	-0.26	0.59	-0.32	1.35	-0.23
	48	4.53	+0.47	1.49	-0.23	0.82	+0.26	0.66	+0.26	1.35	-0.06
0.01	12	4.54	-1.14	2.12	+0.01	0.81	-0.50	0.45	-0.33	1.16	-0.26
	24	4.78	-0.78	2.21	+0.19	0.74	-0.31	0.57	-0.34	1.25	-0.34
	48	3.55	-0.55	1.68	-0.04	0.38	-0.18	0.46	+0.06	1.09	-0.32
0.02	12	3.78	-1.95	1.36	-0.77	0.63	-0.69	0.63	-0.15	1.17	-0.25
	24	6.14	+0.66	3.13	+1.13	1.02	-0.03	0.71	-0.20	1.27	-0.31
	48	5.53	+1.52	2.65	+0.95	0.76	+0.20	0.79	+0.39	1.33	-0.08
0.04	12	4.79	-0.88	2.54	+0.44	0.67	-0.64	0.58	-0.20	0.99	-0.44
	24	2.20	-3.51	0.72	-1.33	0.49	-0.57	0.33	-0.59	0.66	-0 93
	48	1.93	-2.24	1.00	-0.73	0.16	-0.40	0.20	-0.20	0.56	-0.86
Solvent control (acetone 0.2%)	12	5.62	-	2.11	-	1.30	-	0.78	-	1.42	-
	24	5.52	-	2.02	-	1.05	-	0.91	-	1.58	-
	48	4.08	-	1.72	-	0.56	-	0.40	-	1.41	-

FIG. 2. DOSE RESPONSE OF PHORATE FOR MITOTIC INDEX

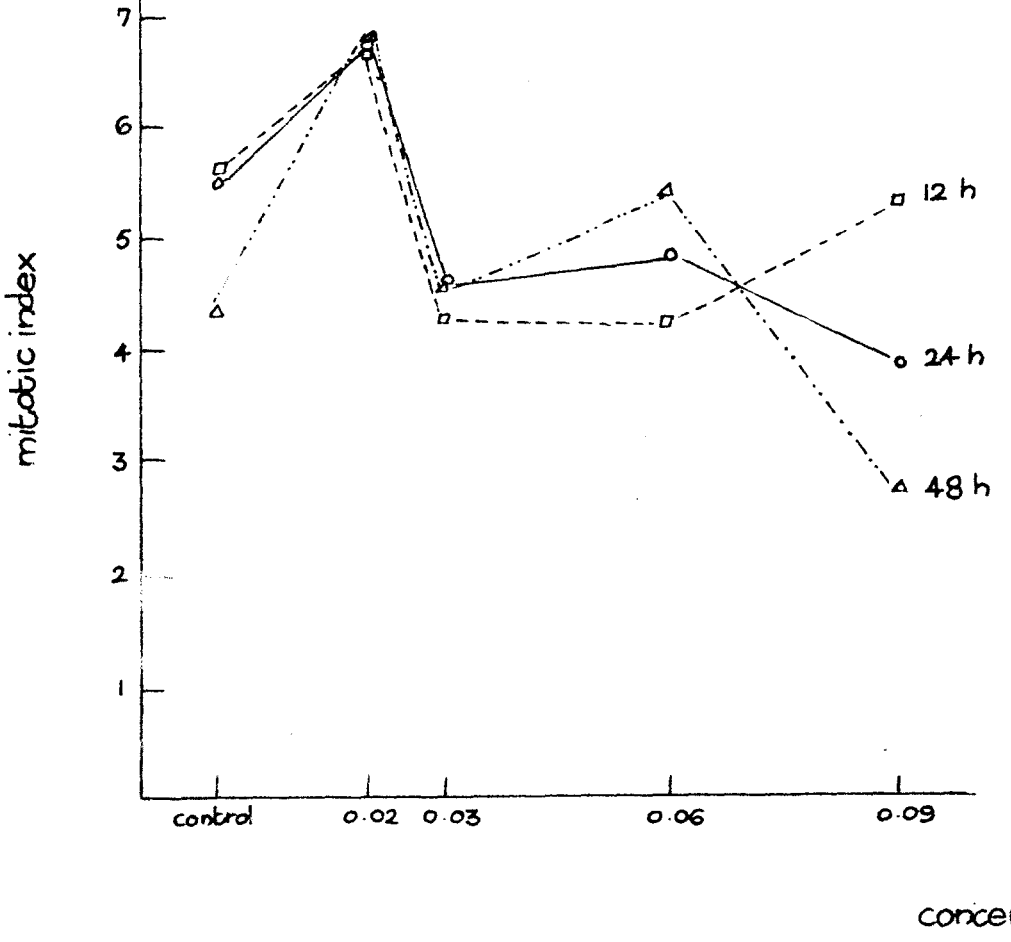
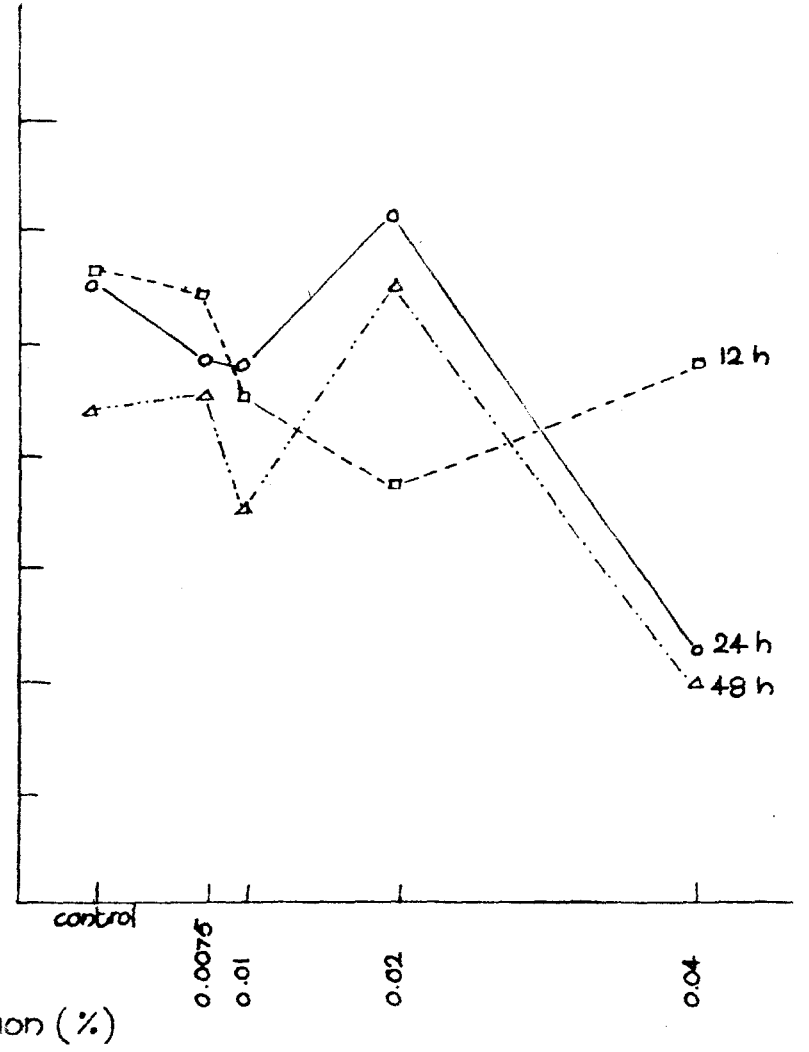


FIG. 3. DOSE RESPONSE OF CARBOFURAN FOR MITOTIC INDEX



dose response curve for mitotic index with different concentration of carbofuran treatment is given in Fig.2.

3. Phorate

The different doses of phorate used for treatment in this study were 0.02, 0.03, 0.06 and 0.09 percentages. The data on mitotic effects are given in Table 5. At the lowest concentration, that is, 0.02 per cent a consistant increase in mitotic index in comparison to the control was observed for all the three treatment durations. This increase can be confirmed from the relative cell division rate given in Table 6, which shows positive values. When the division phases were analysed for their indices, it was found that while the prophase indices recorded a consistant increase, the metaphase indices exhibited reduction in comparison to the control. The metaphases with 12 and 24 hours treatments showed relative cell division values of -0.11 and -0.22 respectively.

An increased concentration of 0.03 per cent decreased the mitotic index initially to 4.38 and 4.60 at 12 and 24 hours of treatments respectively. With 48 hours treatment the value was 4.57 which was almost comparable to the control.

Table 5. Cytotoxic effects of different concentrations of phorate in Allium cepa, L.

Concentration (percentage)	Treatment period (hours)	Total number of cells examined	No.of dividing cells	Mitotic index	Stages of division							
					Prophase		Metaphase		Anaphase		Telophase	
					Total	Index	Total	Index	Total	Index	Total	Index
0.02	12	3023	204	6.75	86	42.16	36	17.65	28	13.73	54	26.47
	24	3366	226	6.71	94	41.59	28	12.39	34	15.04	70	30.97
	48	2829	194	6.85	94	48.45	27	13.92	21	10.82	52	26.80
Mean of treatment periods		-	-	6.77	-	44.07	-	14.65	-	13.20	-	28.08
0.03	12	3564	156	4.38	63	40.38	35	22.44	18	11.54	40	25.64
	24	3089	142	4.60	76	53.52	10	7.04	20	14.08	36	25.35
	48	4271	195	4.57	77	39.49	30	15.38	26	13.33	62	31.79
Mean of treatment periods		-	-	4.52	-	44.46	-	14.95	-	12.98	-	27.59
0.06	12	2348	102	4.34	43	42.16	12	11.76	12	11.76	35	34.31
	24	2575	128	4.97	42	32.81	24	18.75	15	11.72	47	36.79
	48	2736	149	5.45	73	48.99	25	16.78	19	12.75	32	21.48
Mean of treatment periods		-	-	4.92	-	41.32	-	15.76	-	12.08	-	30.86
0.09	12	2655	143	5.39	62	43.36	24	16.78	15	10.49	42	29.37
	24	3419	131	3.83	59	45.04	17	12.98	17	12.98	38	29.00
	48	2471	69	2.79	28	40.58	13	18.84	9	13.04	19	27.54
Mean of treatment periods		-	-	4.00	-	42.99	-	16.20	-	12.17	-	28.64
Solvent control (acetone)	12	3455	194	5.62	73	37.63	45	23.20	27	13.92	49	25.26
	24	3607	201	5.57	73	36.32	38	18.91	33	16.42	57	28.36
	48	3771	154	4.08	65	42.21	21	13.64	15	9.74	53	34.42
Mean of treatment periods		-	-	5.09	-	38.72	-	18.58	-	13.36	-	29.35

The mitotic indices with 0.06 per cent phorate treatment for 12 and 24 hours were 4.34 and 4.97; with 48 hours treatment, it was increased to 5.45 from the corresponding 4.08 of the control. All the stages of division except telophase showed increase in their respective indices in comparison to control.

The highest concentration, 0.09 per cent, recorded a general decline in the mitotic index. Though with 12 hour treatment the value was 5.39, prolonged periods of treatments of 24 and 48 hours tended to drastically reduce the mitotic index to 3.83 and 2.79 respectively. The response of different concentrations at different treatment periods are represented graphically in Fig.2.

A comparison of mitotic effects of all the levels of aldrin, carbofuran and phorate were made by means of histograms in Fig.4.

B. CHROMOSOME ABNORMALITIES

The clastogenicity and other chromotoxic effects of the insecticides were scored from well spread stages of cell division. About 100 to 150 cells of each phase from each treatment were examined for abnormalities. The normal cell division stages are shown in Plate 1a, b, c and d. The abnormalities observed with each

Table 6. Relative division rate of cells and mitotic phase in *Allium cepa*, L. treated with phorate.

Concentration (percentage)	Treatment period (hours)	Percentage of dividing cells	Relative cell division rate (RDR)	Stages of division							
				Prophase		Metaphase		Anaphase		Telophase	
				Percentage	RDR	Percentage	RDR	Percentage	RDR	Percentage	RDR
0.02	12	6.75	+1.15	2.84	+0.75	1.19	-0.11	0.93	+0.15	1.79	+0.38
	24	6.71	+1.26	2.79	+0.79	0.83	-0.22	1.01	+0.10	2.08	+0.51
	48	6.85	+2.89	3.32	+1.62	0.95	+0.39	0.74	+0.34	1.84	+0.44
0.03	12	4.38	-1.31	1.77	-0.35	0.98	-0.32	0.51	-0.27	1.12	-0.30
	24	4.60	-0.97	2.46	+0.45	0.32	-0.74	0.65	-0.26	1.17	-0.42
	48	4.57	+0.51	1.80	+0.08	0.70	+0.20	0.61	+0.21	1.45	+0.04
0.06	12	4.34	-1.35	1.83	-0.29	0.51	-0.80	0.51	-0.27	1.49	+0.07
	24	4.97	-0.58	1.63	-0.40	0.93	-0.12	0.58	-0.33	1.83	+0.25
	48	5.45	+1.43	2.67	+0.97	0.91	+0.35	0.69	+0.29	1.17	-0.24
0.09	12	5.39	-0.24	2.34	+0.23	0.90	-0.41	0.56	-0.22	1.58	+0.16
	24	3.83	-1.79	1.73	-0.30	0.50	-0.56	0.50	-0.41	0.77	-0.82
	48	2.79	-1.34	1.13	-0.60	0.53	-0.03	0.36	-0.04	1.84	+0.44
Solvent control (acetone 0.2%)	12	5.62	-	2.11	-	1.30	-	0.78	-	1.42	-
	24	5.52	-	2.02	-	1.05	-	0.91	-	1.58	-
	48	4.08	-	1.72	-	0.56	-	0.40	-	1.41	-

FIG. 4 COMPARISON OF MITOTIC INDICES

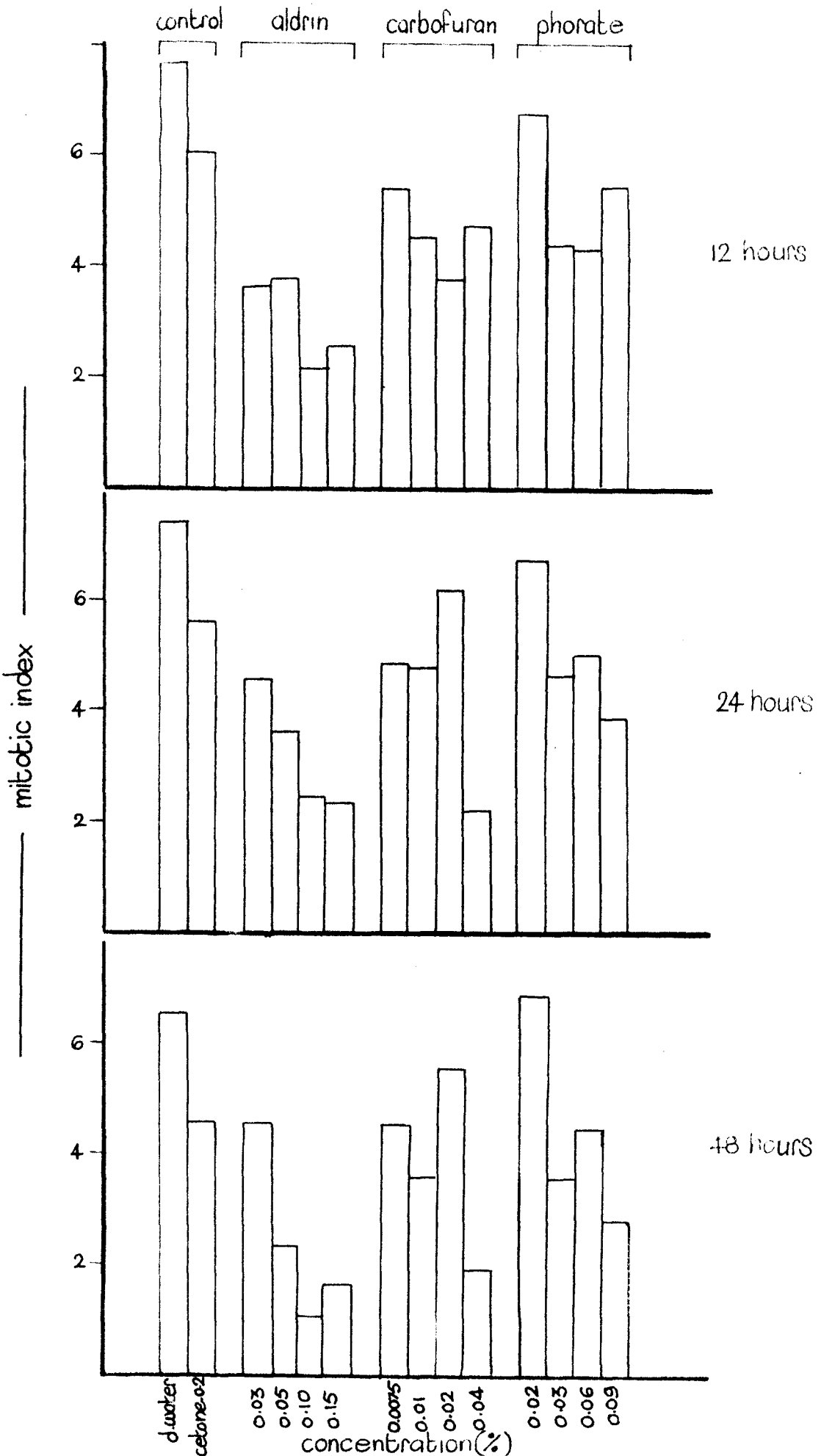


Plate 1 Normal mitotic cell division stages in *Allium cepa*, L.

a. Prophase

b. Metaphase

c. Anaphase

d. Telophase

Plate 1a

Plate 1b

Plate 1d

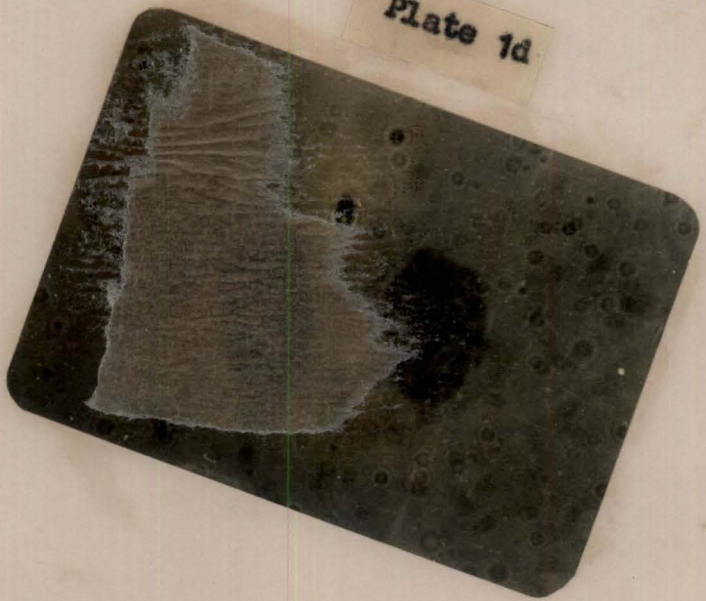


Plate 1c

insecticide as well as the controls are given below.

In the control, where roots of onion bulbs were grown in distilled water for 12, 24 and 48 hours, various abnormalities to the extent of 0.68 per cent, 0.88 per cent and 0.71 per cent respectively were recorded. The abnormalities were expressed in the form of stickiness of chromosomes in prophase and chromosome bridges and laggards in anaphase cells.

In the solvent control with 0.2 per cent acetone, the total abnormalities observed were 1.89, 3.69 and 4.46 percentages at 12, 24 and 48 hours of treatment respectively. These abnormalities induced stickiness, non-orientation, lagging and bridging of chromosomes to varying levels at different periods of treatment.

1. Aldrin

The frequency of different types of abnormalities after aldrin treatment are given in Table 7. The percentage of abnormality ranged from 3.24 to 25.25 in various concentrations and periods of treatments. The total abnormalities in relation to the various concentrations are graphically presented in Fig.5. The concentrations of aldrin had a direct relationship on induction of chromosomal abnormalities.

FIG. 5. CHROMOTOXIC RESPONSE OF ALDRIN

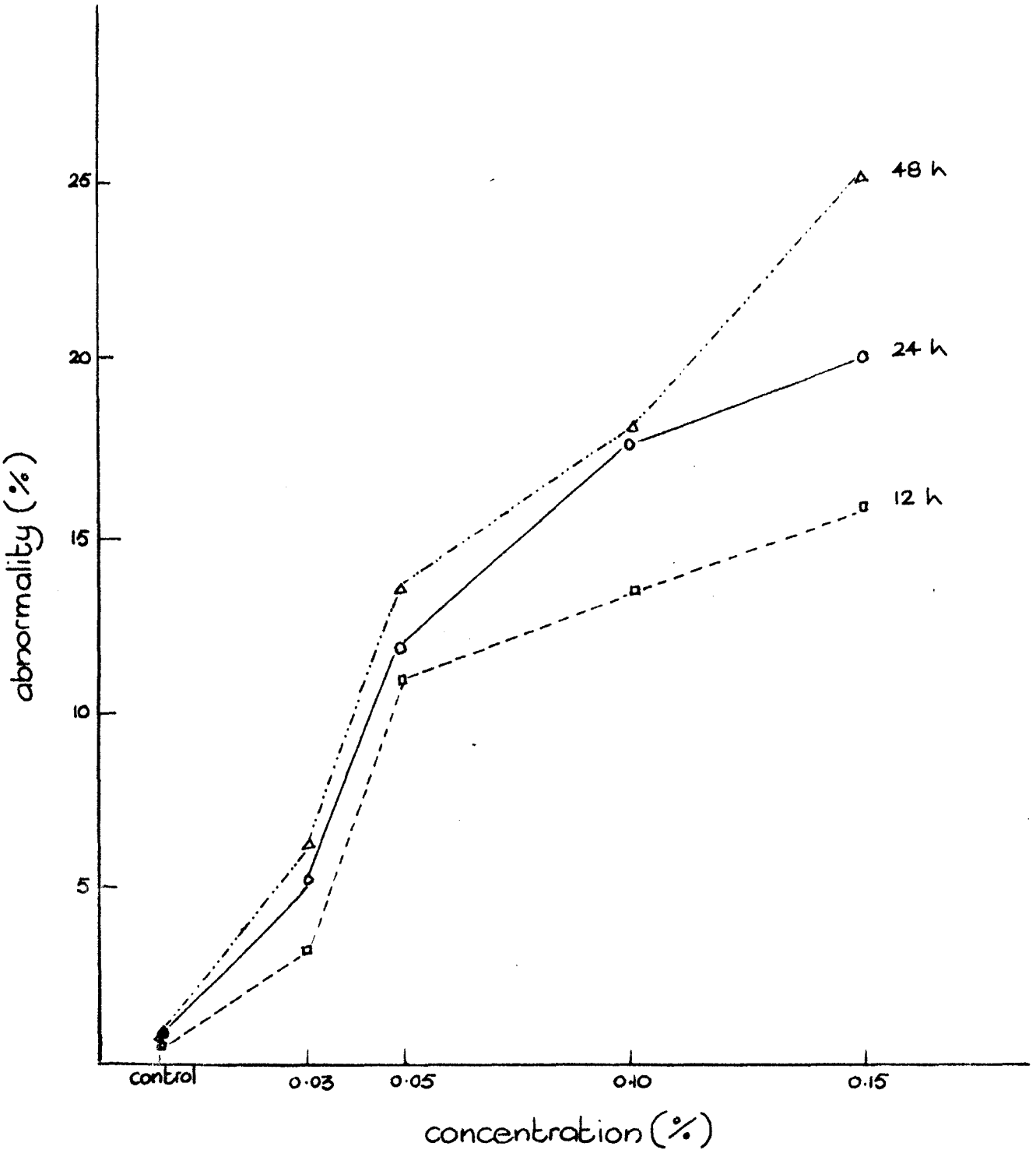


Table 8. Chromosomal abnormalities induced by different concentrations of aldrin in prophase cells of *Allium cepa*, L.

Concentration (percentage)	Treatment period (hours)	Stickiness	Strays		Blurred chromosome	Total abnormalities	Total cells examined
			Single	Double			
0.03	12	2 (1.23)	0	0	0	2 (1.23)	162
	24	5 (2.99)	0	0	2 (1.20)	7 (4.19)	167
	48	7 (6.31)	0	0	1 (0.90)	8 (7.21)	111
0.05	12	10 (11.36)	2 (2.27)	0	2 (2.27)	14 (15.90)	88
	24	7 (7.00)	0	0	2 (2.00)	9 (9.00)	100
	48	10 (7.25)	0	0	5 (3.62)	15 (10.87)	138
0.10	12	9 (7.50)	2 (1.67)	1 (0.83)	4 (3.33)	16 (13.33)	120
	24	12 (8.63)	1 (0.72)	1 (0.72)	8 (5.76)	22 (15.83)	139
	48	10 (14.93)	0	0	2 (2.99)	12 (17.91)	67
0.15	12	11 (6.88)	0	0	4 (2.50)	15 (9.38)	160
	24	12 (19.35)	0	0	3 (4.84)	15 (24.19)	62
	48	15 (24.19)	2 (3.23)	0	5 (8.06)	22 (35.48)	62
Total		110 (7.99)	7 (0.51)	2 (0.15)	38 (2.76)	157 (11.41)	1376

(Contd.)

Table 8 (Contd.)

Concentration (percentage)	Treatment period (hours)	Stickiness	Strays		Blurred chromosome	Total abnormalities	Total cells examined
			Single	Double			
Control (distilled water)	12	1 (0.78)	0	0	0	1 (0.78)	129
	24	1 (0.77)	0	0	0	1 (0.77)	130
	48	2 (1.69)	0	0	0	2 (1.69)	118
Total		4 (1.06)	0	0	0	4 (1.06)	377

Figures given in parentheses indicate percentage

Table 9. Chromosomal abnormalities induced by different concentrations of aldrin in metaphase cells of *Allium cepa*, L.

Concentration (percentage)	Treatment period (hours)	Stickiness	Break		Nonoriented chromosome			Star metaphase	C-metaphase	Blurred chromosome	Total aberrations	Total cells examined
			Single	Multiple	Single	Double	Multiple					
0.03	12	5 (4.10)	0	0	0	0	0	0	0	0	5 (4.10)	122
	24	4 (3.67)	0	0	2 (1.83)	0	0	1 (0.92)	0	0	7 (6.42)	109
	48	3 (2.97)	0	0	1 (0.99)	0	0	0	0	0	4 (3.96)	101
0.05	12	5 (3.94)	2 (1.57)	1 (0.79)	1 (0.79)	0	1 (0.79)	1 (0.79)	0	0	11 (8.66)	127
	24	6 (6.82)	1 (1.14)	0	2 (2.27)	0	0	1 (1.14)	0	0	10 (11.36)	88
	48	8 (7.27)	0	0	4 (3.64)	0	0	2 (1.81)	0	0	14 (12.73)	110
0.10	12	7 (6.19)	2 (1.77)	0	2 (1.77)	1 (0.88)	0	2 (1.77)	1 (0.88)	2 (1.77)	17 (15.04)	113
	24	8 (8.33)	0	0	2 (2.08)	1 (1.04)	1 (1.04)	1 (1.04)	0	0	13 (13.54)	96
	48	6 (8.82)	0	0	2 (2.94)	1 (1.47)	0	3 (4.41)	1 (1.47)	0	13 (19.12)	68
0.15	12	12 (10.00)	0	0	3 (2.50)	0	0	2 (1.67)	0	5 (4.17)	22 (18.33)	120
	24	3 (4.54)	1 (1.52)	0	3 (4.54)	0	0	0	2 (3.03)	2 (3.03)	11 (16.67)	66
	48	7 (7.22)	0	0	5 (5.15)	0	0	0	1 (1.03)	1 (1.03)	14 (14.43)	97
Total		74 (6.08)	6 (0.49)	1 (0.08)	27 (2.22)	3 (0.25)	2 (0.16)	13 (1.07)	5 (0.41)	10 (0.82)	141 (11.59)	1217

(Contd.)

Table 9 (conted.)

Concentration (percentage)	Treatment period (hours)	Stickiness	Break		Nonoriented chromosome			Star metaphase	C-metaphase	Blurred chromosome	Total aberrations	Total cells examined
			Single	Multiple	Single	Double	Multiple					
Control (distilled water)	12	0	0	0	0	0	0	0	0	0	0	95
	24	0	0	0	0	0	0	0	0	0	0	90
	48	0	0	0	0	0	0	0	0	0	0	96
Total		0	0	0	0	0	0	0	0	0	0	281

Figures given in parentheses indicate percentage

Aldrin at 0.03 per cent induced 3.24, 5.30 and 6.22 per cent abnormalities with 12, 24 and 48 hours treatments. The different types of aberrations recorded were stickiness (Plate 5) nonorientation of chromosomes in equatorial plate, laggards (Plate 18), anaphase bridges (Plates 10 and 11) precocious movement of chromosomes in anaphase, micronuclei (Plate 21) etc. At a higher concentration, that is, 0.05 per cent, these aberrations were recorded with higher frequencies. The percentages of aberrations were increased considerably to 11.11 at 12 hours of 0.05 per cent concentration from 6.22 at 48 hours of 0.03 per cent of aldrin. Blurred borders of chromosome, irregular movement during anaphase and chromosome break were also found in few cells (Plates 2, 14, 4). The chromatin bridge (Plate 20) and chromatin bodies were regular types of abnormalities noted in aldrin treatment. The 0.10 per cent concentration was capable of inducing C-metaphase cells (Plate 7) though to a lesser percentage of 0.25 and 0.36 at 12 and 48 hours in addition to other types of abnormalities. Very rarely unequal sized nuclei were formed (0.36%) in this concentration. The total abnormalities showed an increasing trend although manifested in varied forms. Stray chromosomes in prophase were also seen in 12 and 24 hours treatment; 0.73 per cent of beaked telophase nuclei were observed in 48 hours of treatment.

Plate 2 Prophase of *Allium cepa* having chromosomes with blurred borders.

Plate 3 Biprophase in *Allium cepa*.

Plate 4 Prophase of *Allium cepa* showing broken chromosomes.

Plate 5 Metaphase of *Allium cepa* showing sticky chromosomes.

Plate 6 Metaphase of *Allium cepa* showing end to end fusion of chromosomes.

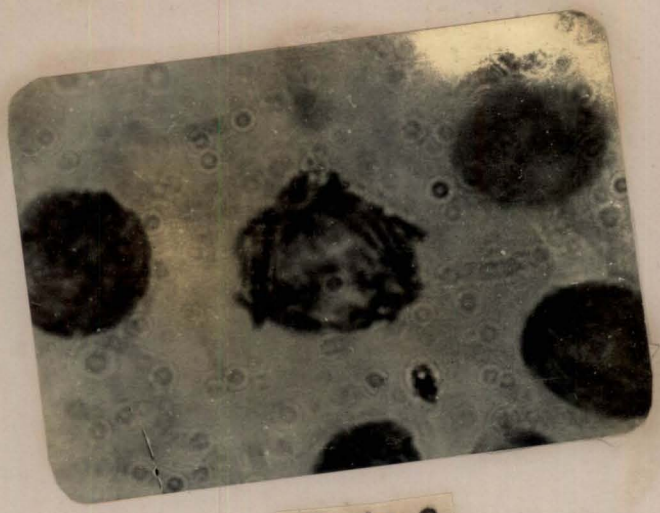


Plate 2

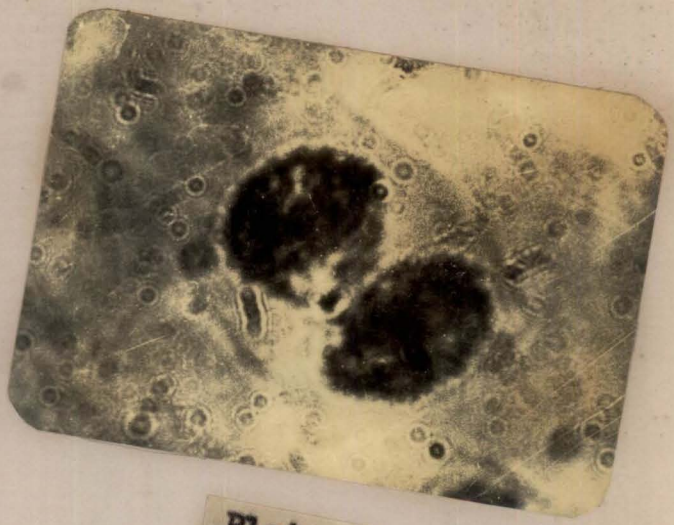


Plate 3

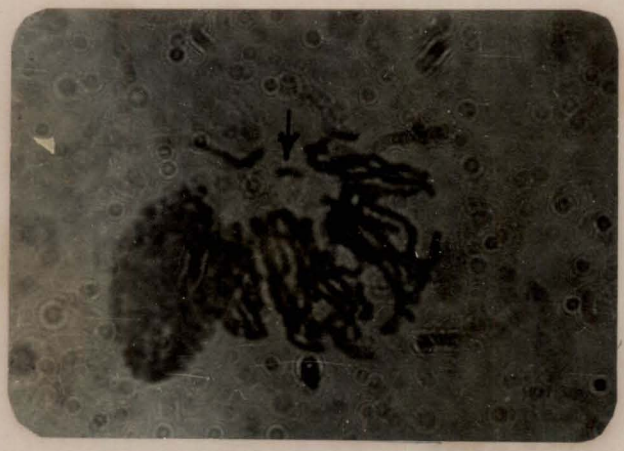


Plate 4

Plate 5

Plate 6

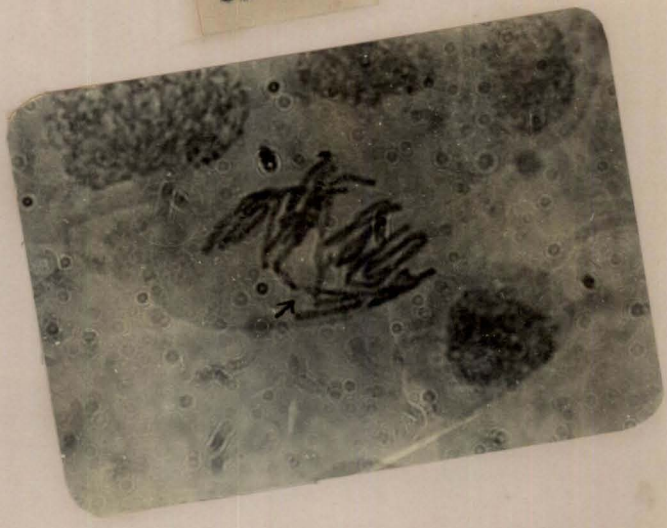


Table 10. Chromosomal abnormalities induced by different concentrations of aldrin in anaphase cells of *Allium cepa*, L.

Concentration (percentage)	Treatment period (hours)	Stickiness	Bridge			Laggard			Precocious movement of chromosome		Irregular anaphase	Total aberrations	Total cells examined
			Single	Double	Multiple	Single	Double	Multiple	Single	Double			
0.03	12	0	3 (2.19)	1 (0.73)	0	1 (0.73)	2 (1.46)	0	1 (0.73)	1 (0.73)	0	9 (6.57)	137
	24	2 (1.64)	2 (1.64)	1 (0.82)	1 (0.82)	0	0	2 (1.64)	0	0	0	8 (6.56)	122
	48	0	2 (2.13)	0	1 (1.06)	0	0	1 (1.06)	1 (1.06)	0	0	5 (5.32)	94
0.05	12	0	5 (5.81)	0	0	1 (1.16)	0	0	0	2 (2.33)	1 (1.16)	9 (10.47)	86
	24	4 (4.12)	3 (3.09)	1 (1.03)	0	4 (4.12)	0	0	3 (3.09)	0	3 (3.09)	18 (18.56)	97
	48	5 (5.26)	2 (2.11)	3 (3.16)	0	5 (5.26)	0	0	2 (2.11)	0	3 (3.15)	20 (21.05)	95
0.10	12	2 (2.63)	3 (3.94)	2 (2.63)	1 (1.32)	1 (1.32)	2 (2.63)	0	2 (2.63)	0	0	13 (17.11)	76
	24	5 (5.32)	3 (3.19)	2 (2.13)	0	5 (5.32)	0	0	3 (3.19)	0	4 (4.26)	22 (23.40)	94
	48	0	4 (5.97)	0	0	3 (4.48)	0	0	1 (1.49)	0	2 (2.99)	10 (14.93)	67
0.15	12	3 (3.26)	4 (4.35)	2 (2.17)	1 (1.09)	2 (2.17)	0	3 (3.26)	0	0	4 (4.35)	19 (20.65)	92
	24	0 (	2 (4.65)	0	0	2 (4.65)	0	0	2 (4.65)	0	2 (4.65)	8 (18.60)	43
	48	0	1 (1.56)	1 (1.56)	1 (1.56)	2 (3.13)	0	5 (7.81)	3 (4.69)	1 (1.56)	2 (3.13)	16 (25.00)	64
Total		21 (1.97)	34 (3.19)	13 (1.22)	5 (0.47)	26 (2.44)	4 (0.37)	11 (1.03)	18 (1.69)	4 (0.37)	21 (1.97)	157 (14.71)	1067

Table 10 (Contd.)

Concentration (percentage)	Treatment period (hours)	Stickiness	Bridge			Laggard			Precocious movement of chromosome		Irregular anaphase	Total aberrations	Total cells examined
			Single	Double	Multiple	Single	Double	Multiple	Single	Double			
Control (distilled water)	12	0	0	2 (2.22)	0	0	0	0	0	0	0	2 (2.22)	90
	24	0	0	2 (2.17)	0	0	0	0	0	0	0	2 (2.17)	92
	48	0	0	1 (1.02)	0	0	0	0	0	0	0	1 (1.02)	98
Total		0	0	5 (1.78)	0	0	0	0	0	0	0	5 (1.78)	280

Figures given in parentheses indicate percentage

Table 11. Chromosomal abnormalities induced by different concentrations of aldrin in telophase cells of *Allium cepa*, L.

Concentration (percentage)	Treatment period (hours)	Micronucleus	Bridge		Laggard	Beaked nucleus	Unequal nucleus	Chromatin bridge	Chromatin body	Total abnormalities	Total cells examined
			Single	Double	Single						
	12	0	2 (1.49)	0	0	0	0	0	0	2 (1.49)	134
0.03	24	2 (1.54)	1 (0.77)	0	0	0	0	2 (1.54)	1 (0.77)	6 (4.62)	130
	48	2 (1.79)	1 (0.89)	0	0	1 (0.89)	0	3 (2.68)	2 (1.79)	9 (8.04)	112
	12	3 (2.65)	2 (1.77)	0	2 (1.77)	0	0	3 (2.65)	2 (1.77)	12 (10.62)	113
0.05	24	3 (2.73)	2 (1.82)	0	0	0	0	2 (1.82)	3 (2.73)	10 (9.09)	110
	48	4 (3.85)	1 (0.96)	0	1 (0.96)	0	0	3 (2.88)	3 (2.88)	12 (11.54)	104
	12	3 (3.41)	2 (2.27)	1 (1.14)	1 (1.14)	0	0	1 (1.14)	0	8 (9.09)	88
0.10	24	5 (5.15)	3 (3.09)	0	0	2 (2.06)	0	4 (4.12)	4 (4.12)	18 (18.56)	97
	48	4 (5.48)	3 (4.11)	0	0	2 (2.74)	1 (1.37)	5 (6.85)	0	15 (20.55)	73
	12	5 (5.68)	2 (2.27)	0	3 (3.41)	1 (1.14)	0	4 (4.55)	2 (2.27)	17 (19.32)	88
0.15	24	2 (2.99)	4 (5.97)	0	2 (2.99)	1 (1.49)	0	4 (5.97)	1 (1.49)	14 (20.90)	67
	48	5 (6.10)	5 (6.10)	0	1 (1.22)	2 (2.44)	1 (1.22)	8 (9.76)	3 (3.66)	25 (30.49)	82
Total		38 (3.17)	28 (2.34)	1 (0.08)	10 (0.84)	9 (0.75)	2 (0.17)	39 (3.26)	21 (1.75)	148 (12.35)	1198

(Contd.)

Table 11 (Contd.)

Concentration (percentage)	Treatment period (hours)	Micronucleus	Bridge		Laggard	Beaked nucleus	Unequal nucleus	Chromatin bridge	Chromatin body	Total abnormalities	Total cells examined
			Single	Double	Single						
	12	0	0	0	0	0	0	0	0	0	125
Control (distilled water)	24	0	0	0	1 (0.71)	0	0	0	0	1 (0.71)	140
	48	0	0	0	0	0	0	0	0	0	111
Total		0	0	0	1 (0.27)	0	0	0	0	1 (0.27)	376

Figures given in parentheses indicate percentage

Plate 7 C-metaphase of *Allium cepa*.

Plate 8 Metaphase of *Allium cepa* showing multiple chromosome abnormalities.

Plate 9 Metaphase of *Allium cepa* showing chromosome fragments.

Plate 10 Anaphase of *Allium cepa* showing single chromosome bridge.

Plate 11 Anaphase of *Allium cepa* showing double chromosome bridge.

Plate 7

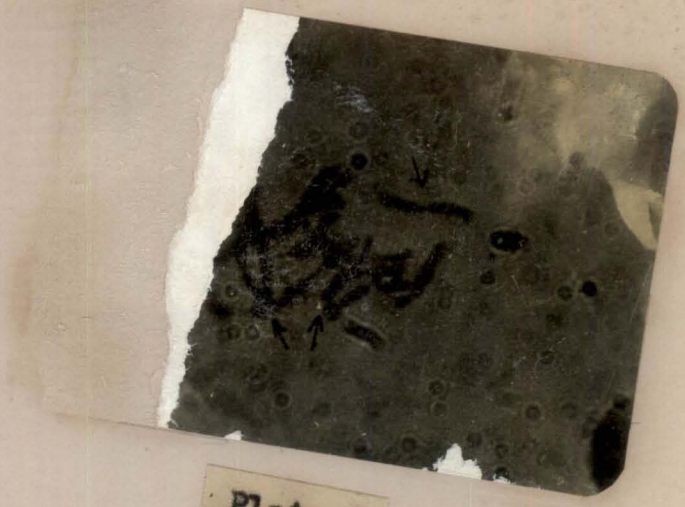


Plate 8

Plate 9

Plate 10

Plate 11

Highest concentration of 0.15 per cent increased the anomalies to 15.87 per cent, 20.17 per cent and 25.25 per cent at 12, 24 and 48 hours respectively and the total frequency of irregularities were significant at both 1 and 0.1 per cent levels of chi square values. The stickiness, laggards and bridges constituted the major proportion of total abnormality at this dosage. An analysis of the manifestation of aberrations in different mitotic phases showed that the maximum frequency of aberration viz., 14.71 per cent, were in the anaphase. This was followed by telophase (12.35%) metaphase (11.59%) and prophase (11.41%). The types and distribution of chromosome aberrations in different division phases are presented in Tables 8, 9, 10 and 11.

2. Carbofuran

Carbofuran induced various types of chromosome abnormalities like stickiness, haziness, nonorientation of metaphase chromosomes, breaks, laggards, bridges and C-metaphases. Their frequency at various concentrations of carbofuran treatment for various time intervals is presented in Table 12. The dose response curve of carbofuran induced chromotoxicity is presented in Fig.6. Aberrations induced at 0.0075 per cent and 0.01 per cent

Tab[illegible]ies induced by different concentrations

Concentration (percentage)	Treatment period (hours)	[illegible]ti-lar [illegible]phase	Micronucleus	Condensed chromosome	Total aberration	Total cells examined	Mean of treatment periods
0.0075	22	[illegible]	0	0	10 (2.31)	433	8.75 (2.92)
	24	[illegible]	0	0	12 (2.44)	492	
	48	[illegible]	0	0	16 (4.00)	400	
0.01	12	0	0	0	20 (3.62)	552	16.91 (5.64)
	24	[illegible]	0	0	27 (4.98)	442	
	48	[illegible]	1 (0.24)	0	32 (8.31)	385	
0.02	12	0	0	1 (0.23)	28** (6.68)	419	24.20 (8.16)
	24	[illegible] 24)	2 (0.48)	0	40** (9.68)	413	
	48	0	0	0	38 (8.60)	442	
0.04	12	0	0	0	30** (7.92)	379	24.56 (8.19)
	24	0	0	0	32* (7.17)	446	
	48	0	0	0	39* (9.18)	425	
Total		[illegible] 02)	3 (0.06)	1 (0.02)	324 (6.20)	5228	
Solvent control (acetone 0.2%)	12	0	0	0	10 (1.89)	530	10.04 (3.35)
	24	0	0	0	20 (3.69)	542	
	48	0	0	0	25 (4.46)	561	
Total		0	0	0	55 (3.37)	1633	

[illegible]rcentage

Table 13. Chromosomal abnormalities induced by different concentrations of carbofuran in prophase cells in *Allium cepa*, L.

Concentration (percentage)	Treatment period (hours)	Stickiness	Breaks			Strays		Haziness	Total aberrations	Total cells examined
			Single	Double	Multiple	Single	Double			
0.0075	12	4 (3.15)	0	0	0	0	0	0	4 (3.15)	127
	24	4 (2.48)	0	0	0	0	0	0	4 (2.48)	161
	48	5 (4.39)	0	0	0	0	0	1 (0.88)	6 (5.26)	114
0.01	12	2 (1.28)	0	0	0	0	0	1 (0.64)	3 (1.92)	156
	24	3 (2.10)	0	0	0	0	0	1 (0.70)	4 (2.80)	143
	48	5 (4.76)	0	0	0	0	0	4 (3.81)	9 (8.57)	105
0.02	12	0	0	0	0	0	0	4 (3.33)	4 (3.33)	120
	24	2 (1.72)	0	0	0	1 (0.86)	1 (0.86)	8 (6.90)	12 (10.34)	116
	48	4 (3.57)	0	0	0	0	0	3 (2.68)	7 (6.25)	112
0.04	12	2 (2.08)	0	1 (1.04)	1 (1.04)	0	0	3 (3.13)	7 (7.29)	96
	24	5 (3.31)	1 (0.66)	0	0	2 (1.32)	0	3 (1.99)	11 (7.28)	151
	48	5 (2.92)	0	0	0	4 (2.34)	0	5 (2.92)	14 (8.19)	171
Total		41 (2.61)	1 (0.06)	1 (0.06)	1 (0.06)	7 (0.45)	1 (0.06)	33 (2.10)	85 (5.41)	1572

(Contd.)

Table 13 (Contd.)

Concentration (percentage)	Treatment period (hours)	Stickiness	Breaks			Strays		Haziness	Total aberrations	Total cells examined
			Single	Double	Multiple	Single	Double			
Solvent control (acetone 0.2%)	12	3 (1.90)	0	0	0	0	0	0	3 (1.90)	158
	24	5 (3.23)	0	0	0	0	0	2 (1.29)	7 (4.52)	155
	48	7 (3.78)	0	0	0	0	0	2 (1.08)	9 (4.86)	185
Total		15 (3.01)	0	0	0	0	0	4 (0.80)	19 (3.82)	498

Figures given in parentheses indicate percentage

Table 14. Chromosomal abnormalities induced by different concentrations of carbofuran in metaphasecells of *Allium cepa*, L.

Concentration (percentage)	Treatment period (hours)	Stickiness	Breaks		Non-oriented chromosomes			Condensed chromosomes	C-metaphase	Total aberrations	Total cells examined
			Single	Double	Single	Double	Multiple				
0.0075	12	0	0	0	1 (0.95)	1 (0.95)	0	0	0	2 (1.90)	105
	24	0	0	0	2 (1.87)	0	0	0	0	2 (1.87)	107
	48	2 (1.89)	0	0	2 (1.89)	0	0	0	1 (0.94)	5 (4.72)	106
0.01	12	3 (2.75)	1 (0.92)	0	1 (0.92)	1 (0.92)	0	0	0	6 (5.50)	109
	24	4 (3.96)	1 (0.99)	0	1 (0.99)	1 (0.99)	0	0	0	7 (6.93)	101
	48	6 (8.22)	0	0	0	0	0	0	1 (1.37)	7 (9.59)	73
0.02	12	2 (1.83)	0	0	1 (0.92)	0	0	1 (0.92)	0	4 (3.67)	109
	24	5 (4.75)	2 (1.98)	0	1 (0.99)	0	0	0	2 (1.98)	10 (9.90)	101
	48	5 (5.05)	1 (1.06)	0	1 (1.01)	2 (2.02)	0	0	2 (2.02)	11 (11.11)	99
0.04	12	3 (2.80)	0	1 (0.93)	2 (1.87)	0	0	0	0	6 (5.61)	107
	24	4 (3.28)	0	0	2 (1.64)	1 (0.82)	1 (0.82)	0	0	8 (6.56)	122
	48	6 (11.32)	1 (1.89)	1 (1.89)	1 (1.89)	0	0	0	0	9 (16.98)	53
Total		40 (3.36)	6 (0.5)	2 (0.17)	15 (1.26)	6 (0.5)	1 (0.08)	1 (0.08)	6 (0.50)	77 (6.46)	1192

(Contd.)

Table 14 (Contd.)

Concentration (percentage)	Treatment period (hours)	Stickiness	Breaks		Non-oriented chromosomes			Condensed chromosomes	C-metaphase	Total aberrations	Total cells examined
			Single	Double	Single	Double	Multiple				
Solvent control (acetone 0.2%)	12	1 (0.84)	0	0	2 (1.68)	0	0	0	0	3 (2.52)	119
	24	2 (1.60)	0	0	2 (1.60)	0	0	0	0	4 (3.20)	125
	48	3 (2.75)	0	0	4 (3.67)	0	0	0	0	7 (6.42)	109
Total		6 (1.70)	0	0	8 (2.27)	0	0	0	0	14 (3.97)	353

Figures given in parentheses indicate percentage

Plate 12 Anaphase of *Allium cepa* showing chromosome bridge and break.

Plate 13 Anaphase of *Allium cepa* showing unequal separation of chromosomes.

Plate 14 Anaphase of *Allium cepa* showing irregular movement of chromosomes.

Plate 15 Anaphase of *Allium cepa* showing multipolar seggregation of chromosomes.

Plate 16 Telophase of *Allium cepa* showing persisting chromosome bridge.

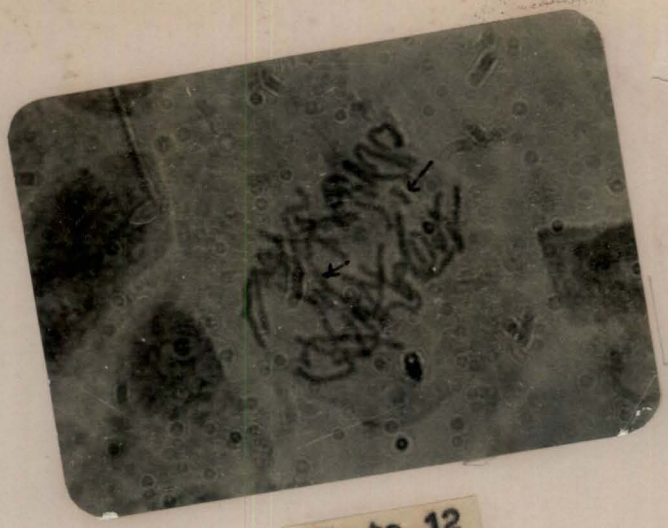


Plate 12

Plate 13

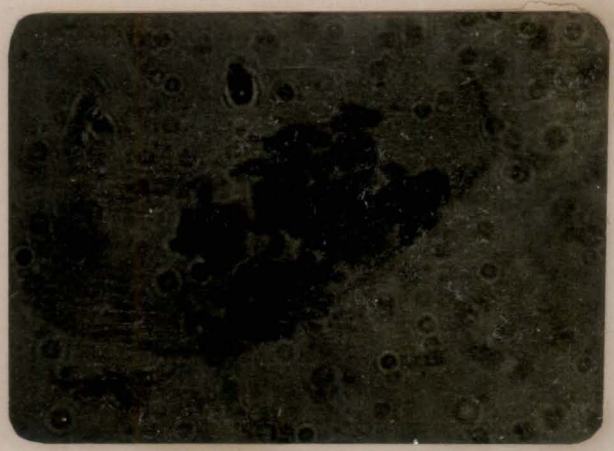


Plate 14

Plate 15

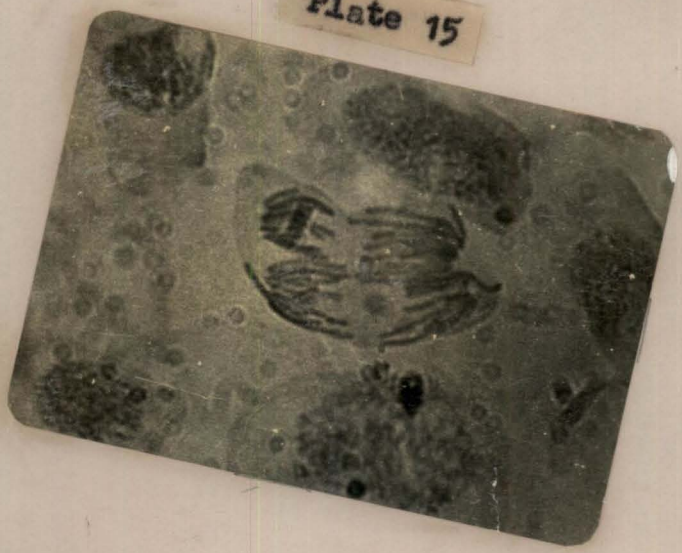


Plate 16

concentrations were not significantly different from the solvent control. However, the higher concentration of 0.02 and 0.04 per cent were very effective in inducing chromosomal abnormalities to significantly higher proportions. The highest aberration frequency was noticed with 0.02 per cent carbofuran treatment for 24 hours. The increase of concentration from 0.02 to 0.04 per cent did not show considerable difference in the frequency of aberrations when means of periods of treatment were compared.

Micronuclei, multipolar anaphase (Plate 15) and chromosome contraction were recorded with frequencies 0.36, 0.24 and 0.23 per cent at 0.02 per cent of carbofuran, the normal field application dosage. Chromosome bridges were very frequent as they were recorded to the tune of 1.86 per cent, which was followed by stickiness (1.55%) and laggards (0.82%), when the total frequency of each aberrant types in carbofuran was considered. Chromosome breaks were noticed in all the mitotic phases (Tables 13, 14, 15 and 16). Single, double and multiple breaks were observed with the treatments of this insecticide. Single, double and multiple chromosome bridges and laggards were also observed. The anaphase stages recorded maximum percentages of aberrations (10.03%), while telophase stages recorded least frequencies of irregularities (3.47%).

Table 15. Chromosomal abnormalities induced by different concentrations of carbofuran in anaphase cells of *Allium cepa*, L.

Concentration (percentage)	Treatment period (hours)	Break		Bridge		Laggards			Multipolar anaphase	Total abnormalities	Total cells examined
		Single	Double	Single	Double	Single	Double	Multiple			
0.0075	12	1 (0.99)	0	1 (0.99)	0	0	0	2 (1.98)	0	4 (3.96)	101
	24	1 (1.00)	1 (1.00)	2 (2.00)	1 (1.00)	1 (1.00)	0	0	0	6 (6.00)	100
	48	0	0	3 (3.00)	0	2 (2.00)	0	0	0	5 (5.00)	100
0.01	12	1 (0.88)	0	3 (2.63)	1 (0.88)	2 (1.75)	1 (0.88)	1 (0.88)	0	9 (7.89)	114
	24	0	0	5 (4.85)	3 (2.91)	2 (1.94)	0	2 (1.94)	0	12 (11.65)	103
	48	2 (2.02)	0	3 (3.03)	2 (2.02)	1 (1.01)	1 (1.01)	0	0	9 (9.09)	99
0.02	12	0	0	8 (7.48)	3 (2.80)	1 (0.93)	0	5 (4.67)	0	17 (15.89)	107
	24	2 (2.20)	1 (1.10)	5 (5.49)	0	2 (2.20)	0	1 (1.10)	1 (1.10)	12 (13.19)	91
	48	2 (1.92)	0	7 (6.73)	3 (2.88)	1 (0.96)	0	0	0	13 (12.50)	104
0.04	12	1 (1.25)	0	3 (3.75)	1 (1.25)	2 (2.50)	4 (5.0)	2 (2.50)	0	13 (16.25)	80
	24	2 (2.27)	0	2 (2.27)	0	2 (2.27)	1 (1.14)	1 (1.14)	0	8 (9.09)	88
	48	1 (1.27)	1 (1.27)	2 (2.53)	4 (5.06)	1 (1.27)	0	0	0	9 (11.39)	79
Total		13 (1.11)	3 (0.26)	44 (3.77)	18 (1.54)	17 (1.46)	7 (0.60)	14 (1.20)	1 (0.09)	117 (10.03)	1166

(Contd.)

Table 15 (Contd.)

Concentration (percentage)	Treatment period (hours)	Break		Bridge		Laggards			Multipolar anaphase	Total abnormalities	Total cells examined
		Single	Double	Single	Double	Single	Double	Multiple			
Solvent control (acetone 0.2%)	12	0	0	2 (1.94)	1 (0.97)	1 (0.97)	0	0	0	4 (3.88)	103
	24	0	0	4 (3.39)	2 (1.69)	2 (1.69)	1 (0.85)	0	0	9 (7.63)	118
	48	0	0	3 (3.03)	2 (2.02)	3 (3.03)	1 (1.01)	0	0	9 (9.09)	99
Total		0	0	9 (2.81)	5 (1.56)	6 (1.88)	2 (0.63)	0	0	22 (6.88)	320

Figures given in parentheses indicate percentage

Table 16. Chromosomal abnormalities induced by different concentrations of carbofuran in telophase cells of *Allium cepa*, L.

Concentration (percentage)	Treatment period (hours)	Break	Bridge		Laggard	Micronucleus	Total aberrations	Total cells examined
		Single	Single	Double	Single			
0.0075	12	0	0	0	0	0	0	100
	24	0	0	0	0	0	0	124
	48	0	0	0	0	0	0	80
0.01	12	0	2 (1.16)	0	0	0	2 (1.16)	173
	24	0	2 (2.11)	1 (1.05)	1 (1.05)	0	4 (4.21)	95
	48	1 (0.93)	3 (2.78)	0	2 (1.85)	1 (0.93)	7 (6.48)	108
0.02	12	0	1 (1.20)	2 (2.41)	0	0	3 (3.61)	83
	24	1 (0.95)	2 (1.90)	1 (0.95)	0	2 (1.90)	6 (5.71)	105
	48	0	4 (3.15)	3 (2.36)	0	0	7 (5.51)	127
0.04	12	0	2 (2.08)	2 (2.08)	0	0	4 (4.17)	96
	24	0	3 (3.53)	0	2 (2.35)	0	5 (5.88)	85
	48	0	5 (4.10)	2 (1.64)	0	0	7 (5.74)	122
Total		2 (0.15)	24 (1.85)	11 (0.85)	5 (0.39)	3 (0.23)	45 (3.47)	1298

(Contd.)

Table 16 (Contd.)

Concentration (percentage)	Treatment period (hours)	Break	Bridge		Laggard	Micronucleus	Total aberrations	Total cells examined
		Single	Single	Double	Single			
Solvent control (acetone 0.2%)	12	0	0	0	0	0	0	150
	24	0	0	0	0	0	0	144
	48	0	0	0	0	0	0	168
Total		0	0	0	0	0	0	462

Figures given in parentheses indicate percentage

FIG. 6. CHROMOTOXIC RESPONSE OF CARBOFURAN

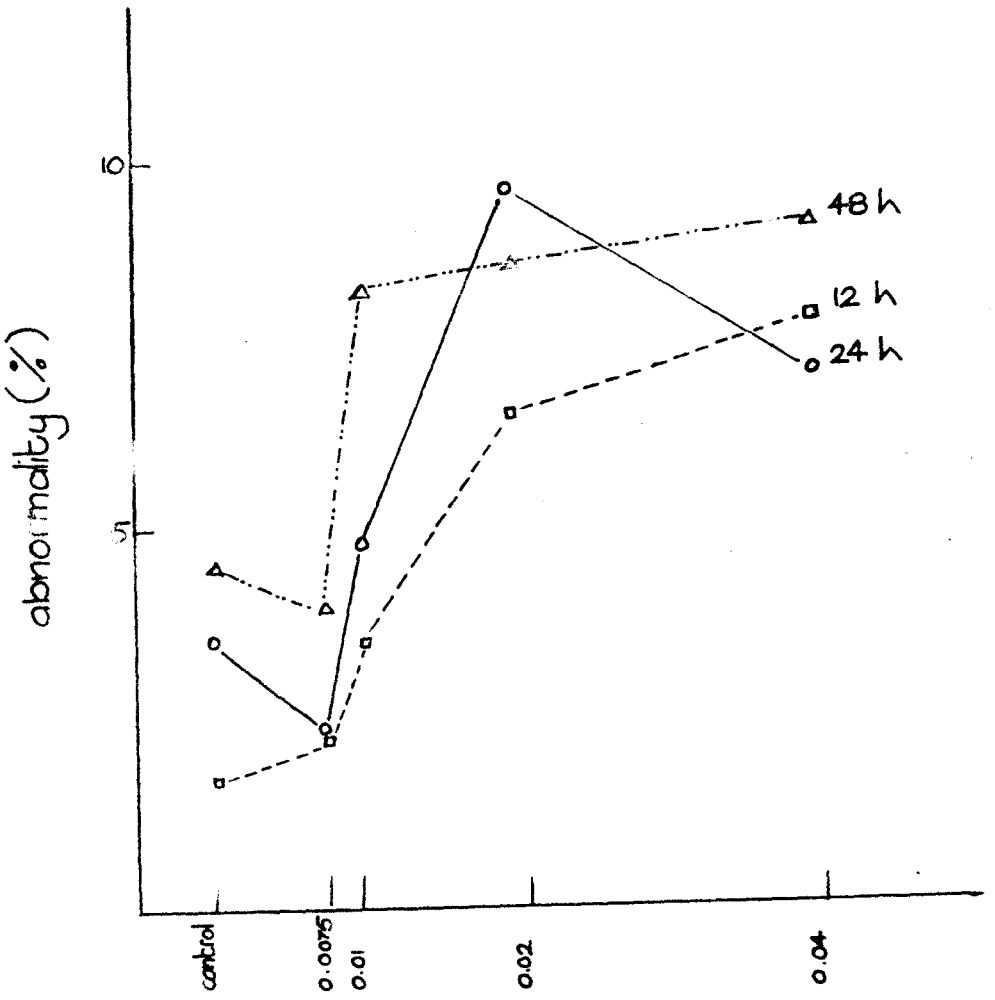


FIG. 7. CHROMOTOXIC RESPONSE OF PHORATE

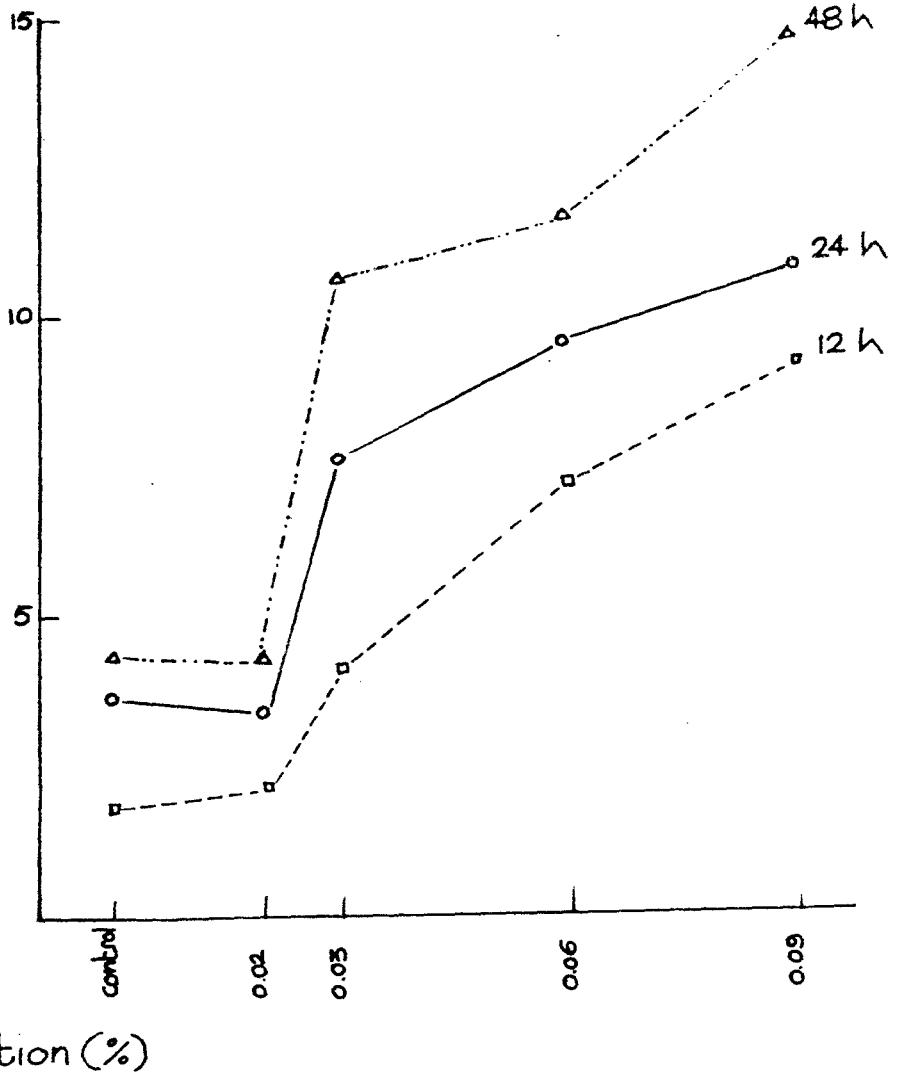


Plate 17 Telophase of Allium cepa showing broken chromosome bridge.

Plate 18 Telophase of Allium cepa showing lagging chromosome.

Plate 19 Telophase of Allium cepa showing precocious movement of chromosome.

Plate 20 Telophase of Allium cepa showing persisting chromatin bridge connections and chromosome fragment.

Plate 21 Telophase of Allium cepa showing micronucleus along with daughter nuclei.

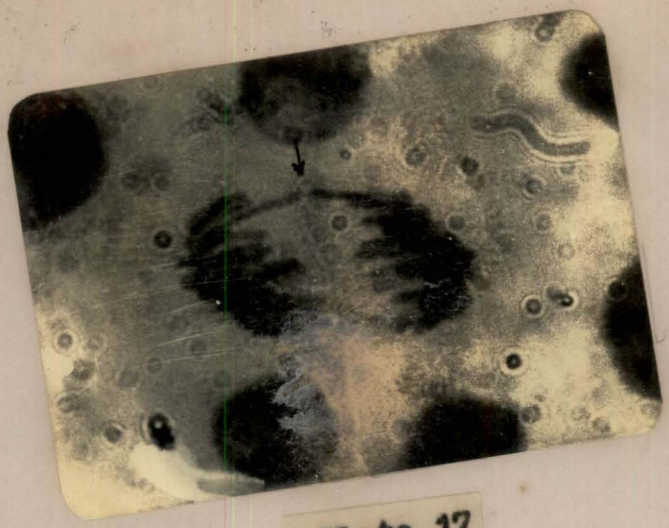


Plate 17

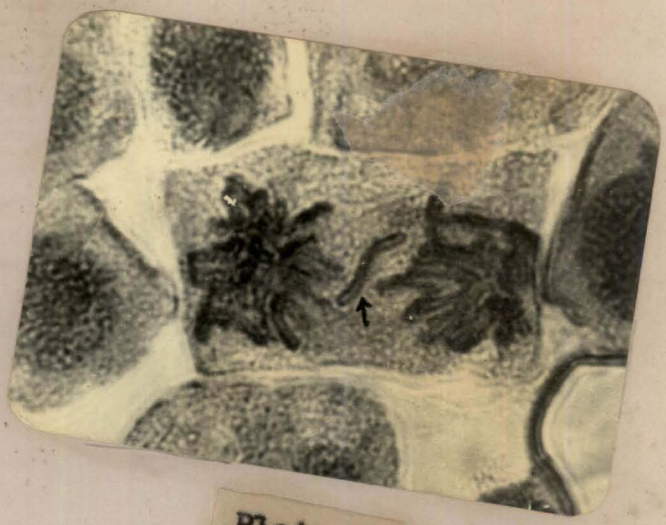
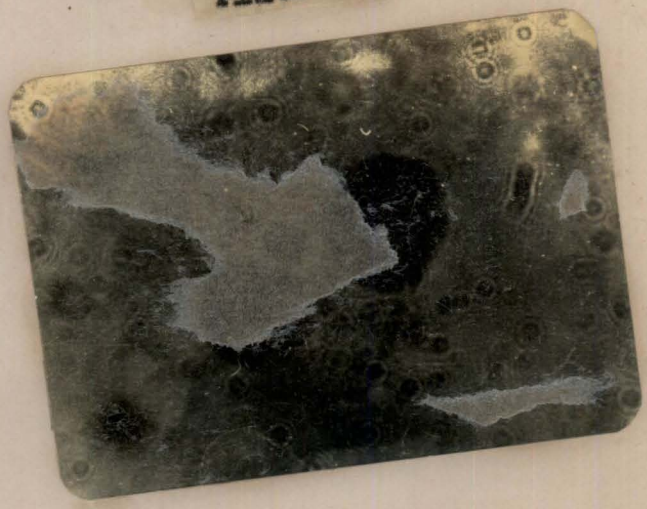


Plate 18

Plate 19

Plate 20

Plate 21



3. Phorate

The frequencies of chromosomal abnormalities induced by 12, 24 and 48 hours treatment with different concentrations of phorate is given in Table 17. Total abnormalities ranged from 2.21 to 14.69 per cent at various dosages. The major anomalies observed in phorate treatment were stickiness, nonorientation of metaphase chromosomes, breaks, laggards, and chromosome bridges. Micronuclei and irregular anaphase were also manifested but to a lesser extent and were usually noted at higher concentrations and at prolonged treatments, while biprophase (Plate 3) and blurred chromosome borders were a very rare phenomenon that occurred occasionally.

The 0.02 per cent concentration, the lowest dosage tried, did not show significant levels of aberrations in all the three treatment periods. The next higher concentration of 0.03 per cent was effective to the significant levels at 24 hours. The increased time interval of 48 hours was much more effective than the 24 hours treatment and it recorded a total abnormality of 10.59 per cent. The two higher doses viz., 0.06 and 0.09 per cent were potential doses for the induction of abnormalities. Both concentration and time of treatment had linear relationship with abnormalities, as shown

normalities induced by different concentrations

Concentration (percentage)	Bridge	Micronucleus	Irregular anaphase	Total aberration	Total cells examined	Mean of treatment period
0.02	2 (0.49)	0	0	9 (2.21)	408	9.88 (3.29)
	4 (0.91)	1 (0.23)	0	15 (3.41)	440	
	5 (1.33)	0	0	16 (4.26)	375	
0.03	11 (2.25)	0	0	20 (4.10)	488	22.28 (7.43)
	10 (2.30)	1 (0.23)	0	33* (7.59)	435	
	18 (3.53)	2 (0.39)	2 (0.39)	54** (10.59)	510	
0.06	16 (3.50)	0	0	33** (7.22)	457	28.22 (9.41)
	13 (3.09)	2 (0.48)	1 (0.24)	40** (9.50)	421	
	16 (2.93)	3 (0.55)	2 (0.37)	63** (11.52)	547	
0.09	16 (3.51)	0	3 (0.66)	42** (9.21)	456	34.76 (11.59)
	20 (4.43)	4 (0.89)	0	49** (10.86)	451	
	15 (3.24)	2 (0.43)	1 (0.22)	68** (14.69)	463	
Total	146 (2.68)	15 (0.28)	9 (0.17)	442 (8.11)	5451	
Solvent control (acetone 0.2%)	3 (0.57)	0	0	10 (1.89)	530	10.04 (3.35)
	6 (1.11)	0	0	20 (3.69)	542	
	5 (0.89)	0	0	25 (4.46)	561	
Total	14 (0.86)	0	0	55 (3.37)	1633	

* indicate percentage
level
level

Table 18. Chromosomal abnormalities induced by different concentrations of phorate in prophase cells of Allium cepa, L.

Concentration (percentage)	Treatment periods (hours)	Stickiness	Break		Haziness	Biprophase	Blurred chromosome	Total abnormalities	Total cells examined
			Single	Double					
	12	1 (0.95)	0	0	0	0	0	1 (0.95)	105
0.02	24	2 (1.85)	1 (0.93)	0	0	0	0	3 (2.78)	108
	48	3 (3.00)	2 (2.00)	0	0	0	0	5 (5.00)	100
	12	1 (0.74)	0	0	0	0	0	1 (0.74)	136
0.03	24	0	1 (0.96)	0	0	1 (0.96)	0	2 (1.92)	104
	48	3 (2.00)	3 (2.00)	0	0	0	2 (1.33)	8 (5.33)	150
	12	2 (1.68)	2 (1.68)	0	0	0	0	4 (3.36)	119
0.06	24	6 (6.38)	2 (2.13)	1 (1.06)	0	0	0	9 (9.57)	94
	48	5 (2.81)	3 (1.69)	2 (1.12)	0	0	3 (1.68)	13 (7.30)	178
	12	1 (0.92)	2 (1.83)	1 (0.92)	1 (0.92)	0	0	5 (4.59)	109
0.09	24	6 (5.41)	2 (1.80)	0	5 (4.50)	0	0	13 (11.71)	111
	48	7 (5.51)	2 (1.57)	0	3 (2.36)	0	1 (0.79)	13 (10.24)	127
Total		37 (2.57)	20 (1.39)	4 (0.28)	9 (0.63)	1 (0.07)	6 (0.42)	77 (5.34)	1441

(Contd.)

Table 18 (Contd.)

Concentration (percentage)	Treatment periods (hours)	Stickiness	Break		Haziness	Biprophase	Blurred chromosome	Total abnormalities	Total cells examined
			Single	Double					
Solvent control (acetone 0.2%)	12	3 (1.90)	0	0	0	0	0	3 (1.90)	158
	24	5 (3.23)	0	0	2 (1.29)	0	0	7 (4.52)	155
	48	7 (3.78)	0	0	2 (1.08)	0	0	9 (4.86)	185
Total		15 (3.01)	0	0	4 (0.80)	0	0	19 (3.82)	498

Figures given in parentheses indicate percentage

Table 19. Chromosomal abnormalities induced by different concentrations of phorate in metaphase cells of *Allium cepa*, L.

Concentration (percentage)	Treatment period (hours)	Stickiness	Break		Nonoriented chromosome		Blurred chromosome	Total abnormalities	Total cells examined
			Single	Double	Single	Double			
0.02	12	2 (1.92)	0	0	0	0	0	2 (1.92)	104
	24	3 (2.44)	0	0	0	0	0	3 (2.44)	123
	48	0	0	0	0	0	0	0	82
0.03	12	0	0	0	0	0	0	0	102
	24	3 (2.65)	2 (1.77)	0	3 (2.65)	0	2 (1.77)	10 (8.85)	113
	48	3 (2.88)	2 (1.92)	1 (0.96)	2 (1.92)	3 (2.88)	0	11 (10.58)	104
0.06	12	5 (4.42)	1 (0.88)	1 (0.88)	1 (0.88)	0	0	8 (7.08)	113
	24	2 (1.79)	3 (2.68)	0	2 (1.79)	0	0	7 (6.25)	112
	48	6 (5.45)	3 (2.73)	2 (1.82)	3 (2.73)	2 (1.82)	0	16 (14.54)	110
0.09	12	5 (4.39)	3 (2.63)	1 (0.88)	1 (0.88)	0	0	10 (8.77)	114
	24	4 (3.48)	2 (1.74)	0	2 (1.74)	0	0	8 (6.96)	115
	48	5 (4.20)	4 (3.36)	3 (2.52)	4 (3.36)	3 (2.52)	3 (2.52)	22 (18.48)	119
Total		38 (2.90)	20 (1.53)	8 (0.61)	18 (1.37)	8 (0.61)	5 (0.38)	97 (7.40)	1311

(Contd.)

Table 19 (Contd.)

Concentration (percentage)	Treatment period (hours)	Stickiness	Break		Nonoriented chromosome		Blurred chromosome	Total abnormalities	Total cells examined
			Single	Double	Single	Double			
Solvent control (acetone 0.2%)	12	1 (0.84)	0	0	2 (0.68)	0	0	3 (2.52)	119
	24	2 (1.60)	0	0	2 (1.60)	0	0	4 (3.20)	125
	48	3 (2.75)	0	0	4 (3.67)	0	0	7 (4.59)	109
Total		6 (1.70)	0	0	8 (2.27)	0	0	14 (3.97)	353

Figures given in parentheses indicate percentage

Table 20. Chromosomal abnormalities induced by different concentrations of phorate in anaphase cells of *Allium cepa*, L.

Concentration (percentage)	Treatment period (hours)	Irregular anaphase	Bridge			Break		Laggard			Total abnormalities	Total cells examined
			Single	Double	Multiple	Single	Double	Single	Double	Multiple		
0.02	12	0	2 (2.02)	0	0	1 (1.01)	0	1 (1.01)	0	0	4 (4.04)	99
	24	0	2 (1.89)	0	0	0	2 (1.89)	2 (1.89)	0	0	6 (5.66)	106
	48	0	1 (1.14)	2 (2.27)	0	1 (1.14)	1 (1.14)	0	0	0	5 (5.68)	88
0.03	12	0	9 (10.23)	0	0	2 (2.27)	0	0	2 (2.27)	3 (3.41)	16 (18.18)	88
	24	0	8 (7.14)	0	0	2 (1.79)	1 (0.89)	1 (0.89)	1 (0.89)	3 (2.68)	16 (14.29)	112
	48	2 (1.67)	10 (8.33)	4 (3.33)	0	3 (2.50)	1 (0.83)	0	0	5 (4.17)	25 (20.83)	120
0.06	12	0	7 (6.48)	2 (1.85)	2 (1.85)	0	0	2 (1.85)	0	3 (2.78)	16 (14.81)	108
	24	1 (0.96)	6 (5.77)	1 (0.96)	3 (2.88)	0	0	1 (0.96)	2 (1.92)	0	14 (13.46)	104
	48	2 (1.74)	4 (3.48)	6 (5.22)	1 (0.87)	4 (3.48)	2 (1.74)	3 (2.61)	2 (1.74)	0	24 (20.87)	115
0.09	12	3 (2.61)	6 (5.22)	1 (0.87)	1 (0.87)	4 (3.48)	2 (1.74)	0	0	0	17 (14.78)	115
	24	0	7 (6.25)	2 (1.79)	5 (4.46)	1 (0.89)	0	2 (1.79)	0	0	17 (15.18)	112
	48	1 (1.00)	8 (8.00)	2 (2.00)	3 (3.00)	4 (4.00)	0	1 (1.00)	2 (2.00)	5 (5.00)	26 (26.00)	100
Total		9 (0.71)	70 (5.52)	20 (1.58)	15 (1.18)	22 (1.74)	9 (0.71)	13 (1.03)	9 (0.71)	19 (1.50)	186 (14.68)	1267

(Contd.)

Table 20 (Contd.)

Concentration (percentage)	Treatment period (hours)	Irregular anaphase	Bridge			Break		Laggard			Total abnormalities	Total cells examined
			Single	Double	Multiple	Single	Double	Single	Double	Multiple		
Solvent control (acetone 0.2%)	12	0	2 (1.94)	1 (0.97)	0	0	0	1 (0.97)	0	0	4 (3.88)	103
	24	0	4 (3.39)	2 (1.69)	0	0	0	2 (1.69)	1 (0.85)	0	9 (7.63)	118
	48	0	3 (3.03)	2 (2.02)	0	0	0	3 (3.03)	1 (1.01)	0	9 (9.09)	99
Total		0	9 (2.81)	5 (1.56)	0	0	0	6 (1.88)	2 (0.63)	0	22 (6.88)	320

Figures given in parentheses indicate percentage

Table 21. Chromosomal abnormalities induced by different concentrations of phorate in telophase cells of *Allium cepa*, L.

Concentration (percentage)	Treatment period (hours)	Break		Bridge		Laggard	Micronecleus	Total abnormalities	Total cells examined
		Single	Double	Single	Double	Single			
0.02	12	2 (2.00)	0	0	0	0	0	2 (2.00)	100
	24	0	0	2 (1.94)	0	0	1 (0.97)	3 (2.91)	103
	48	2 (1.90)	0	2 (1.90)	0	2 (1.90)	0	6 (5.71)	105
0.03	12	0	0	2 (1.23)	0	1 (0.62)	0	3 (1.85)	162
	24	2 (1.89)	0	2 (1.89)	0	0	1 (0.94)	5 (4.72)	106
	48	2 (1.47)	2 (1.47)	4 (2.94)	0	0	2 (1.47)	10 (7.35)	136
0.06	12	0	0	4 (3.42)	1 (0.85)	0	0	5 (4.27)	117
	24	3 (2.70)	0	3 (2.70)	0	2 (1.80)	2 (1.80)	10 (9.00)	111
	48	0	0	4 (2.77)	1 (0.69)	2 (1.38)	3 (2.08)	10 (6.94)	144
0.09	12	2 (1.69)	0	6 (5.08)	2 (1.69)	0	0	10 (8.47)	118
	24	0	0	5 (4.42)	1 (0.88)	1 (0.88)	4 (3.54)	11 (9.73)	113
	48	1 (0.85)	1 (0.85)	2 (1.71)	0	1 (0.85)	2 (1.71)	7 (5.98)	111
Total		14 (0.98)	3 (0.21)	36 (2.51)	5 (0.35)	9 (0.63)	15 (1.05)	82 (5.73)	1432

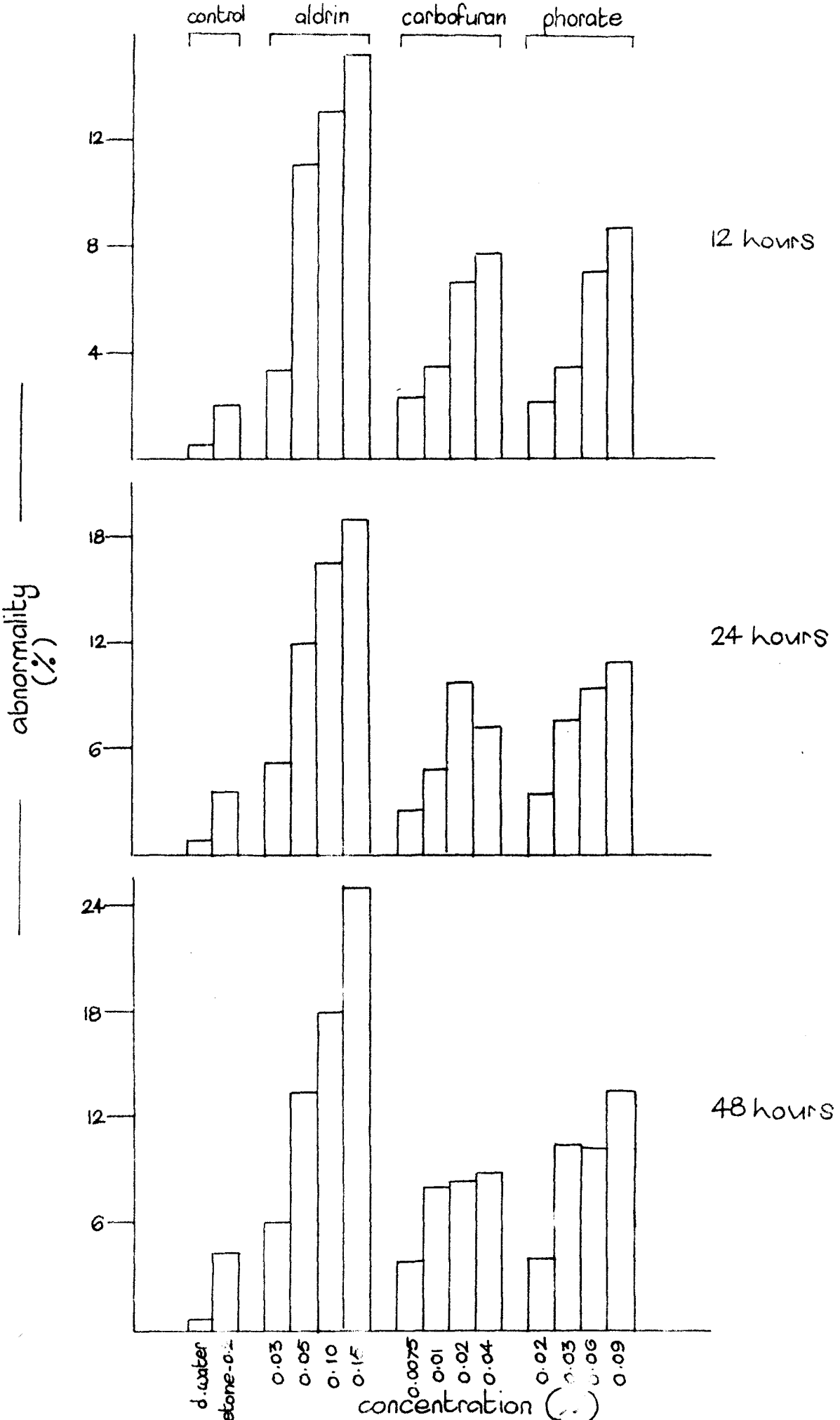
(Contd.)

Table 21 (Contd.)

Concentration (percentage)	Treatment period (hours)	Break		Bridge		Laggard	Micronucleus	Total abnormalities	Total cells examined
		Single	Double	Single	Double	Single			
	12	0	0	0	0	0	0	0	150
Solvent control (acetone 0.2%)	24	0	0	0	0	0	0	0	144
	48	0	0	0	0	0	0	0	168
Total		0	0	0	0	0	0	0	462

Figures given in parentheses indicate percentage

FIG.8. COMPARISON OF CHROMOTOXIC RESPONSES



in Fig.7. Table 17 reveals that chromosome bridges were the most frequent type of abnormality in all the concentrations of phorate. They constituted 2.68 per cent of the total aberrations. Breaks (1.83%), sticky chromosomes (1.38%) and laggards (0.92%) in the decreasing order of occurrence were the other major types of anomalies in Allium cepa root meristem cells after phorate treatment.

An examination of the distribution of chromosomal aberrations in the various stages of mitosis (Tables 18, 19, 20 and 21) revealed that the maximum frequency of abnormalities were manifested during anaphase. The major types of anomalies in this stages were chromosome bridges, chromosome breaks and laggards. Thus, while the frequency of abnormalities during anaphase was 14.68 per cent, the corresponding values were 5.34, 7.40 and 5.73 per cent during prophase, metaphase and telophase respectively.

The chromatoxic effects of four levels of aldrin, carbofuran, and phorate were compared with the control and among themselves at different periods of time by means of histogramsin Fig.8.

Discussion

DISCUSSION

The results of various cytotoxic and clastogenic investigations of three insecticides, viz., aldrin, carbofuran and phorate on root mitosis of Allium cepa, L., are discussed hereunder in the light of akin investigations carried out by different workers.

A. CYTOTOXICITY

The cytotoxicity of any chemical/agent is manifested as total mitodepression, nuclear pycnosis and chromosome clumping (Sahu et al., 1981). The dosage at which these symptoms appear is considered as the cytotoxic concentration. The mitodepressive property of a chemical or an agent can be assessed by the computation of mitotic index in the treated and control experiments. The mitotic index reflects the percentage of dividing cells at any particular stage of development. It may increase or decrease by the influence of external agency acting on it. An increase may lead to an unprecedented multiplication of cells which result in tumor or cancerous out growth in organised tissues of animals, or gall like protrusion in organised tissues of plants or tall and lanky growth of plants if the tissue affected are meristems. A decreased rate of mitotic division compared to control show the

mitodepressive property of the agent leading to retarded or stunted growth of the organism. More severe effect of such chemicals can lead to mitotoxicity - whereby cells in the treated organisms will not be in a position to multiply and grow.

A reduction in mitotic index may mainly be due to (i) disturbance in DNA synthesis during interphase which is a pre-requisite for cell division (ii) disturbance in the synthesis of protein whereby various enzymes necessary for the process of cell division are affected, or (iii) disturbance in the metabolic activities which may lead to the non-availability of energy required for cell division.

1. Aldrin

In the present investigation, the mitotic index was reduced significantly by aldrin treatments. It was reduced to half that of control, in the lowest dose (0.03%) tried. This indicates the chronic effects of aldrin on root mitosis. This negative effect continue further at higher concentrations with a linear relationship of mitodepression and concentration. Such an inhibition of mitotic index by organochlorine pesticides was observed by Scholes (1955) in *Allium cepa* with heptachlor and Bakale *et al.* (1981) in *Malvastrum coromandelianum* with 2, 4-Dichlorophenoxy acetic acid. Mitodepressive effects of DDT, DDD and DDE have been reported by Mahu and Herbet (1975) in mice.

The observation that among the various divisional phases, the frequency of prophase was reduced to maximum with aldrin treatment suggests that the number of cells entering into division was reduced by these treatments. This may point to the possibility that aldrin inhibits the DNA synthesis resulting in defective synthetic phase (S-phase) of interphase rendering many a cells incapable of entering into division. The inhibition of DNA synthesis by organochlorinated insecticides in albino rats has already been reported by Banerjee et al. (1981). They have also found in their study that the amounts of total proteins and lipids are unaffected by the treatment with these chemicals. This can be ascribed as the reason why in the present investigation the drastic reduction in prophase index was not expressed in the indices of other division phases. The cells entering into prophase completes the cell cycle through the rest of the divisional stages, even-though in a low key, with the available enzymes in the cytoplasmic environment of the cell. The period of treatment could not manifest any significant change in mitotic index. The increase of metaphase and anaphase indices, at least in lower doses, may probably be due to the fact that these stages were temporarily arrested as a result of the delay in spindle fibre formation and slow coiling and uncoiling of these fibres at anaphase to carry the

chromosomes to the poles. This suggests that the spindle proteins are not completely free from the effects of aldrin. The lipid and protein taking part in cell wall formation did not seem to have affected much.

2. Carbofuran

Carbofuran has been found to produce marginal decrease in mitotic index compared to the control. However, higher concentration (0.04%) have brought about significant reduction in mitotic index. At lower doses, the marginal reduction in mitotic index recorded with 12 hours treatment was found to get nullified with increase in treatment period at least in 0.02 per cent. This points to the possibility that the chemical is either getting neutralised or the metabolic products are rendered ineffective. However, Ahmed et al. (1977) have reported that N-nitroso derivatives, the degradation product of carbamate, is not completely inactive on DNA. The present observation suggests that carbofuran is not an effective cytotoxicant at lower doses. No definite dose dependant behaviour also is noticed as already reported by Amer and Farah (1974b) in the case of Duphar and IPC, two carbamate insecticides.

The prophase indices showed increased frequency in all the concentrations while the anaphase and telophase

indices decreased. This suggests that either the number of cells entering to division is more than the control or the prophase stage is prolonged by letting the frequency of other divisional stages get reduced. A reduction in anaphase and telophase indices is observed with various treatments. It seems to be reasonable to suggest that the proteins which are constituent of enzymes which trigger the dissolution of nuclear membrane during prophase are enhanced by carbofuran. This may be due to its nitroso - derivative affecting the protein which in turn can be as a result of change caused in the DNA (Morrison, 1962).

The increase in prophase index recorded at the highest concentration tended to show drastic reduction by extending the period of treatment. This may be attributed to the N-nitroso derivative which in sufficient concentration is capable of affecting the DNA as suggested by Ahmed et al. (1977). Banerjee et al. (1981) also have found that the DNA and RNA tend to decrease with carbaryl (a carbamate insecticide) and protein remained constant from their studies on albino rat. The reduction in nucleic acid synthesis might have hindered the mitotic division in higher concentrations. The proteins remain constant and therefore the metaphase and anaphase stages continue at the same rate as the spindle mechanism seem to be unaffected.

3. Phorate

Phorate increased the mitotic index at the lowest dose of 0.02 per cent when compared with the control. At higher concentrations, however, the mitotic index started decreasing and maximum reduction was noted at the highest concentration tried (0.09%). The spread spectrum of indices of mitotic phases showed that increase in mitotic index was shared by all the divisional phases in equal proportions as evidenced by the positive values of relative division rates. Giri et al. (1981) have observed an increase in total DNA, RNA and protein content of albino rats treated with malathion and parathion, two organophosphatic insecticides. Thus, the increase in mitotic index as a result of phorate treatment may be ascribed to be associated with increase in the nucleic acids and protein activity with this chemical.

Higher concentrations of phorate could lead to the toxicity of the insecticide disturbing the internal millieu of the cells and could not permit them to enter into prophase, either by inhibiting DNA synthesis, or the synthesis of other prerequisites of the cell division as opined by Mishra and Sinha (1979) with their studies with malathion, and Panda and Sharma (1980) with trichlorfon and dichlervos in barley meristems. This can be the reason

for a decreasing tedency of the mitotic index by increasing concentrations. The division phases, except telephase, proceeded at the same rate as directed by the prophase, the starter of the division cycle. Telophase was found to increase compared to control which may be attributed to the arrest of this stage by the delay in formation of cell wall indicating the possible effect of phorate on proteins.

The extension of period of treatment have positive response with respect to mitotic index. The mitotic index was low at 12 hours and it improves with 24 and 48 hours of treatment. This implies that the mitodepressive property of the insecticide seen in 12 hours of treatment is a transitory one and high mitotic index values in a longer periods suggest that the metabolite/s of phorate resulting due to its degradation have no mitotoxic properties or even they may have certain amount of stimulatory activity.

B. CHROMOSOME ABNORMALITIES

Chromosome abnormalities have been used as a measure of reproductive successes in plants and have also been correlated with morphological and taxonomical changes, fertility and sterility relationships, mutations and other characteristics (Grant, 1978). In addition, they may lead to lethal mutations in submammalians and microorganisms and deleterious effects on mammalian cells

or to the future generations. The chromosome abnormalities which can be a manifestation of the effects of a particular chemical on the genetic material of an organism, can be taken as a parameter to know whether the chemical is clastogenic. In general, chromosome aberrations may provide both qualitative and quantitative data on the effects of exposure to mutagens or any such agents which can prove detrimental not only to the organism *per se* but to its future generations as well (Grant, 1978).

1. Aldrin

The chlorinated hydrocarbon pesticides are noted for their persistance following application and widespread disruption in the ecosystem. From the foregoing results it is apparent that treatments of *Allium cepa* L., root tips with aldrin produces a high percentages of chromosomal abnormalities. It is clear from the data, that the effects were dose responsive. The aberrations effected even at the lowest concentration tried significantly varied from control.

The most common and frequent anomaly was stickiness of chromosomes. This abnormality had a linear relationship with dose. This was observed to the tune of 4.22 per cent in the lowest dose tried and was spread on prophase,

metaphase and anaphase chromosomes. Klasterska et al. (1976) opined that chromosome stickiness arises from the improper folding of the chromosome fibre into single chromatids and chromosomes. As a result there is an intermingling of fibres, and the chromosome become attached to each other. Stephan (1979) suggested that stickiness is due to stripping of protein from the chromosomes. Induction of sticky chromosomes by carbaryl treatment was reported earlier by Amer (1965) in Allium cepa and Johnson and Jalal (1973) in mice. 2, 4-D and 2, 4,5-T were also found to be capable of producing stickiness of chromosomes, in broad bean (Amer and Ali, 1974). Depolymerisation of nucleic acids leading to stickiness and clumping of chromosome has been reported by Sterrett and Fretz (1975) in onion root tip cells treated with asulum, an organochlorinated herbicide.

The stickiness of chromosomes is further prolonged to metaphase as well, even though with a lower frequency. The reduction in the frequency may be due to partial rectification of the anomalies caused to the chromosome structure and function by aldrin treatment. As the division proceeds through anaphase and telophase, the frequency of this chromosome abnormality is further reduced. In these two stages these are manifested as chromosome bridges,

either due to simple sticking or due to the fusion of broken ends of chromosome. Three types of bridges were observed in the present investigation: single, double and multiple. The frequency of single bridges was more than 75 per cent, while that of multiple bridges were less than 6 per cent. Amer and Ali (1974) reported bridges in Vicia faba on treatment with 2, 4-D and 2,4,5-T and Mohandas and Grant (1972) in Hordeum vulgare treated with 2, 4-D. At telophase the chromosome bridges may ultimately break and broken ends may be withdrawn into the nuclei, which show a pear shaped or beak like projection on the nuclei (beaked nuclei). Some of the organochlorinated compounds like para-dichlorobenzene are reported to be capable of producing such abnormal nuclei in mitotic telophase cells of Lens esculentum (Sharbhoy, 1980). Higher concentrations of aldrin is found to induce this abnormality in Allium cepa, though the frequency is not very high.

Extreme stickiness of chromosomes which persists even after the completion of the cell cycle is manifested in the form of massive thick connection between two chromatin bodies. Such chromatin bridges have been reported earlier by Tanaka (1956) in Tradescantia paludosa by natural radiation and by Srivastava (1966) in para-dichlorobenzene treated Vicia faba cells.

Occasionally the prophase as well as metaphase chromosomes appear with blurred border probably due to their partial dissolution. Jagoda (1980) reported such anomalies in Allium cepa when treated with a herbicide, simazine. This may again point to the possibility that aldrin is capable of acting on the chromosomal proteins, both histones and nonhistones.

Lagging chromosomes were a regular phenomenon observed with the various treatments of aldrin. These are chromosomes which lag or move slowly to the poles during anaphase. Chromosome lagging can be due either to the partial dysfunctioning of spindle fibres or to the inactive centromere of these chromosomes. This is indicative of the possible effect of aldrin on spindle protein as well as chromosomal protein and/or DNA. Such a possibility of action of 2,4-D in different weed plants (Tomkins and Grant, 1976) and endrin in barley (Singh et al., 1977) have been reported earlier. Bakale and Hadke (1981) also observed laggards in a weed species (Euphorbia geniculata) treated with 2,4-D and Lasso, the two weedicides. In the present study, the third major type of chromosomal abnormality was of this type.

Break in the chromosomes was a rare phenomenon noted in aldrin treatment. The chromosome break is

generally considered to involve DNA molecule. The DNA modifying property of some of the chlorinated hydrocarbon pesticides is reported by McCoy et al. (1978) and Pal et al. (1981). Another possible mechanism thought to be responsible for chromosome breakage with chemicals is the inhibition of biochemical process of catalysis of DNA molecules. Insecticides may destroy or enhance synthesis of certain enzymes (Wuu and Grant, 1966). A five-fold increase in the frequency of breaks with pesticides was reported in human cells by Yoder et al. (1973). Markaryan (1967) with aldrin and heptachlor and Bhojvaid and Vijayakumar (1981) with endosulfan observed chromosome breaks in bone marrow cells of mice.

The acentric fragments resulting from chromosome breaks as well as the lagging chromosomes normally do not move to either of the poles in pace with the rest of the chromosomes and therefore get excluded from the daughter nuclei and form micronuclei or restitution nuclei other than the normal nuclei. This is evidenced by the regular occurrence of such structure in aldrin treated telophase cells. Thus the micronuclei are the chromosome residues of spindle dysfunction as evidenced by some of the abnormalities and breaks (Sharma and Sahu, 1977; Sahu et al., 1981; Subhash and Rajam, 1983). The chromosome or chromosome fragments in these micronuclei in the next

divisional stage normally appear as stray chromosomes away from the normal complement of prophase chromosomes. This phenomenon also has been noticed in some of the treatments in the present investigation.

The metaphase chromosomes showed various types of abnormalities in aldrin treated cells. One of them was the nonorientation of chromosomes at the equaterial plate at early metaphase. Barthelmess (1957) opined that the phenomenon of nonorientation of chromosomes due to irregular prometaphase movement was accompanied by adhesion of centromere to the slowly dissolving nuclear membrane or the surrounding plasma. The single chromosome nonorientation was more than 84 per cent of the total nonorientation (including double and multiple chromosomes nonorientation). This type was found in all the concentrations. A nonfunctional centromere also can be attributed to be the reason for nonorientation of that chromosomes in the equatorial plate.

Star metaphases were another abnormality regularly occurring in these treatments. The chromosomes may or may not divide longitudinally but they meet and aggregate in the centre of the cell with their kinetechore being adhered together at the same point giving a star like appearence to the chromosome complement. Amer, 1965 observed

this type of abnormality in Sevin treated Vicia faba mitotic cells.

As reported earlier in case of some of the organochlorinated insecticides (Wuu and Grant, 1967; Datta, 1966; Banerjee et al., 1981) aldrin also is found to be capable of inducing C-mitotic metaphases. This may be due to the complete inactivation of the spindle mechanism and concommitant disturbance of the chromosome movement indicating the possible action of aldrin on the spindle proteins. The impairment of spindle mechanism is further suggested by irregular anaphase and precocious movement of chromosomes recorded in this experimentations as reported earlier by Deysson (1968). The action of the chemical is comparable to the phenomenon reported by Mercykutty and Stephan (1980) with adriamycin in Allium cepa cells. The irregularity and precocity in the movement of chromosome during anaphase has probably led to the formation of unequal sized telophase nuclei which was observed in the treatments. Thus, this phenomenon may lead to the production of aneuploid cells and associated anomalies as suggested by Deysson, 1968.

Chromatin bodies, a less frequent aberration in this treatment, which appear as aggregated or coalescent mass of chromosomes of various sizes were observed in the

late telophase and interphase cells. This might be due to the disintegration of chromosomes into a chromatin mass. This type of abnormality was recorded by Wuu and Grant (1967) in barley meiosis treated with Lorox. This phenomenon is indicative of the dysfunctioning of enzymes by biologically active organochlorinated compounds as suggested by Mohandas and Grant (1972) and Shaw and Garner (1983).

2. Carbofuran

Carbofuran also showed clastogenic properties, though to a lower extent, in comparison to aldrin. The maximum frequency of chromosome abnormalities were recorded as chromosome bridges and this was followed by the various other anomalies like stickiness, laggards, haziness, breaks, nonorientation of chromosomes, starys and C-metaphase in their decreasing order and also very rare phenomenon like micronuclei, multipolar anaphase and chromosome contraction. These observations indicate the possible action of carbofuran on the DNA and protein in living cells as reported earlier in case of various other carbamate insecticides by different workers (Parry, 1973; Ahmed et al., 1977; Elevins et al., 1977; Cheng and Conner, 1982).

Haziness of chromosomes in late prophase is referred to the long thin threads of chromosomes resulting from

their partial despiralisation probably due to ineffective proteinaceous binding. Such effects of carbamate insecticides like chloropropham, propham and monuron have already been observed in barley (Herichova, 1970; and Wuu and Grant, 1967). Stickiness and the resultant sticky chromosomes further indicate the inactivation of chromosomal proteins. Stickiness of chromosomes was a common type of irregularity found in prophase and metaphase. The stickiness might have led to the formation of anaphase bridges as suggested by Amer and Farah (1974a; 1976).

Effect of carbofuran on the chromosomal protein is further suggested by the appearance of chromosome contraction due to change in the normal chromosome coiling. Extreme coiling as the result of malfunctioning of the protein associated with chromosome can be the major reason for this type of abnormality. Carbamate insecticides like IPC have been found to be capable of inducing such abnormalities in various test systems (Storey and Mann, 1967) and Amer and Farah, 1974b).

The carbamates induced breaks in chromosomes as indicated in the present study. It was found in all the concentrations tried. This property of different carbamate insecticides have been reported earlier by various workers (Wuu and Grant, 1966; 1967; Prasad and Parmer, 1968;

Tomkins and Grant, 1976 and Ahmed et al., 1977). The breaks were recorded in all the stages, single breaks being more frequent than double and multiple breaks. The breaks persisted in the anaphase stages too. Laggards probably originating from the broken accentric fragments or chromosomes with and defective centromeres were also a frequent abnormality in all the treatments. The incidence seems to be dose dependant. Occurrence of laggards have been reported earlier in Vicia faba and Gossypium barbadense (Amer and Farah, 1974 a) and in wheat (Al-Najjar and Soliman, 1980) with carbamate insecticide treatments. These laggards and accentric fragments most of the times take the shape of micronuclei during telophase as seen in few of the treatments in this study. Occurrence of micronuclei in Vicia faba induced by Duphar has been reported by Amer and Farah (1974b).

Chromosome breaks expose their sticky ends which are capable of fusing together to give rise to bridges during their anaphase movement. Such anaphase bridges also were observed with all the doses of carbofuran treatment. This again was dose dependant suggestive of their origin from the broken chromosomes. Chromosome breaks and bridges as well as the probable action of carbamates have been discussed earlier by various authors (Amer and Farah, 1976; Al-Najjar and Soliman, 1980 and Soliman and Al-Najjar, 1980).

3. Phorate

Phorate is found to induce higher proportions of chromosome aberrations compared to carbofuran. The effects of phorate was dose as well as time responsive exhibiting a linear relationship between these two and the frequency of abnormalities produced. The abnormalities noted were bridges, breaks, stickiness, laggards, nonorientation of chromosomes in equatorial plate, micronuclei, blurred chromosome border, haziness, irregular anaphase and biprophase in their decreasing order of occurrence.

Stickiness of chromosome, as reported earlier by Amer and Farah (1979) in Vicia faba is found to be induced by the various treatments with phorate in Allium cepa in the present investigation. Chromosome breaks were also observed in all the treatments and the frequency was definitely dose dependant. It was the second largest type of aberration. The organophosphorus insecticides are found to bring about alkylation of bases of DNA strand, primarily guanine (Lofroth et al., 1969; Panda and Sharma, 1980). Bridges breaks induced by various organophosphorus insecticides have been reported in different plant systems (Amer and Farah, 1979; Singh et al., 1977).

Stickiness of chromosome as well as broken chromosome might have led to the formation of chromosome bridges which

was also a regular abnormality recorded in the various phorate treatments. Induction of chromosome bridges by some of the organophosphorus compounds like trichlorgon, dichlorvos, Dimecron-100, Rogar-40 etc. have been reported earlier (Reddy and Rao, 1969; Amer and Farah, 1979; Panda and Sharma, 1980). These authors have also observed the existance of laggards probably resulting from chromosome breaks. The acentric fragments or the chromosomes with nonfunctional centrumeres usually form the laggards. This causes a definite change in the genetic make up of the daughter cells. In the present study, different concentrations of phorate is found to be capable of induction of laggards, both in metaphase and anaphase though no definite relationship between the frequency of breaks and laggards could be envisaged.

Reddy and Rao (1969) have reported that Dimecron-100 and Rogar-40 could induce various spindle abnormalities in barley. However, phorate is found to have very little effect, if at all it has, on the spindle proteins. This becomes evident from the lack of C-metaphase and other abnormalities associated with a defective spindle mechanism of dividing cells. A small frequency of irregular anaphases were, however, observed in this study indicating that the action of phorate on spindle proteins cannot be ruled out

altogether. The occurrence of biprophase in one of the treatments, probably due to the lack of cytokinesis in the proceeding division also is suggestive of the possible action of this chemical on proteins.

Micronuclei are the resultant products of laggards as suggested by Amer and Farah (1979). Dose and duration of treatment responsive induction of micronuclei is observed in the experiment.

The present investigation on the cytotoxicity and clastogenicity of aldrin, carbofuran and phorate suggests that the insect toxicity levels (the commonly used field doses) cannot be considered completely safe on the cellular constituents of the organisms coming in contact. All the three chemicals tested, could act on the proteins and also the genetic material, DNA, though to different magnitudes, as evidenced by their effect on mitotic index as well as the various types of chromosomal abnormalities induced. While aldrin is found to be the most potent mitodepressive and clastogenic compound, the other chemicals were also not safe at their field doses. The results obtained indicate the potential genetic danger associated with the three chemicals by their indiscriminate and careless handling.

In case of carbofuran and phorate, the lowest concentrations tried, viz., 0.0075 and 0.02 per cent

respectively can be considered as safe doses in the present test system. However, with regard to aldrin, 0.03 per cent, which is much below the field dose also has been found to induce significant cytotoxic and clastogenic potentiality suggesting that a very low per cent of the chemical reaching in the cytoplasmic millieu of cells can hamper with their normal functioning and lead to changes in the genetic make up of these cells.

An extrapolation of the results obtained in this study to higher animals and human beings may not be an easy task. However, it will be worthwhile cautioning about the potential dangers associated with the indiscriminate and careless handling of these chemicals as well as their far reaching implication on human welfare.

Summary

SUMMARY

While the value of pesticides in agriculture production is immense, the unscientific and indiscriminate use of them has lead to many secondary unwanted and/or deleterious consequences in the biological environment. Some of them are known to be cytotoxic, carcinogenic, teratogenic, clastogenic and mutagenic by the works of various investigators. Considering the various genetic potentialities of these compounds, the present investigation was carried out to assess the cytotoxicity and clastogenicity of three very commonly used insecticides, viz., aldrin, carbofuran and phorate and also to find out cytogenetically safe levels of these chemicals using Allium cepa, L. root tip assay.

The concentrations of these insecticides were fixed taking into consideration of their field dose of application in insect control. Four concentrations of each insecticides were used for this study. The treatments were given for different durations such as 12, 24 and 48 hours. The squash preparations of treated root tips were made using feulgen stain for studying mitotic index and chromosomal abnormalities.

The mitotic index was computed from about 4000 cells in each treatment. Indices of each stages of mitotic division were also computed. The chromosome abnormalities were scored from well spread stages of cell division. About 100 to 150 cells of each phases of division for each treatment were scanned for this.

Aldrin was found to be highly mitodepressive in the present test system. It was dose responsive. The inhibition of DNA synthesis was reflected as reduction in prophase index and it also affected the spindle protein as indicated by the accumulation of metaphase and anaphase cells. Time of treatment could not manifest any significant change in mitosis.

Carbofuran brought about marginal reduction in mitotic index. The present investigation suggested that carbofuran is not an effective toxicant at lower doses. At higher concentrations extension of period of treatment showed a drastic mitodepression compared to control suggesting the effect of byproduct at sufficient concentrations.

Phorate, on the other hand, increased mitotic index in lowest concentrations (0.02%), but at higher concentrations showed decreasing tendency and the reduction was maximum at 0.09 per cent. The longer periods of treatment increased mitotic index, suggesting a stimulatory effect of phorate on metabolites of cell constituents.

While studying the chromosome abnormalities it was observed that aldrin had a direct dose relationship with aberrations. The percentage of aberrations induced ranged from 3.24 to 25.25 per cent. Stickiness of chromosomes was most common type of aberration (4.22%) in prophase and metaphase. These abnormalities were manifested as bridges in anaphase and telophase stages. While stickiness in extreme cases appeared as chromatin bridge, the broken chromosome bridge appeared as beaked nuclei. Lagging of chromosome was the third largest phenomenon. The laggards and breaks gave rise to micronuclei in late telophase and stray chromosomes in next divisional phase. Single, double and multiple non-orientation of chromosomes were noticed in all concentrations at different frequencies. Star metaphase, C-metaphase, irregular anaphase, precocious movement to poles and unequal nuclei were also recorded to a lesser degree in various treatments.

Carbofuran, unlike aldrin, could not induce abnormalities to a significant level in lowest dose (0.0075%) tried compared to control. At higher concentrations chromosome bridges were at maximum frequencies (1.86%). The second largest anomaly recorded was stickiness (1.55%). This was followed by the various anomalies like laggards, haziness, breaks, non-orientation of chromosomes, strays, C-metaphase, micronulei, multipolar anaphase and chromosome

contraction. The maximum frequency of chromosome anomalies (9.68%) were noticed at 24 hours of 0.02 per cent carbofuran. The concentrations 0.02 per cent and 0.04 per cent could not bring out any notable change in abnormalities. The type of abnormalities induced by this insecticide indicated that it affect both nucleic acids and proteins.

As with carbofuran, phorate also could not induce chromotoxicity to significant levels at lowest dose (0.02%). However, it could induce aberrations at higher concentrations. It exhibited a linear relationship with concentrations and period of treatment in inducing aberrations. Bridges were recorded to maximum level (2.68%) followed by breaks (1.83%) and stickiness (1.38%). The laggards, non-orientation of chromosomes in equatorial plate, micronuclei, blurred chromosome border, haziness, irregular anaphase and biprophase were also observed in their decreasing order of occurrence. The anomalies suggested the effect of phorate on DNA and protein.

The present investigation on the cytotoxicity and clastogenicity of aldrin, carbofuran and phorate suggests that the field dose of application cannot be considered safe on the cellular constituents of the organism. In the case of carbofuran and phorate the lowest concentrations tried,

viz., 0.0075 and 0.02 per cent respectively can be considered as safe dose in the present test system. Aldrin at 0.03 per cent, below field dose was found to induce significant mitodepressive and clastogenic effects suggesting its adverse effect on genetic make up of the cells and organism as a whole. All the three insecticides tested seems to have the potentiality to act on the genetic material as well as proteins, at least in higher doses.

References

REFERENCES

Ahmed, M. and Grant, W.F.(1972). Cytological effects of pesticides phosdrin and bladex on Tradescantia and Vicia faba. Can. J. Genet. Cytol., 14 : 157-165.

Ahmed, F.E., Ronald, W.H. and Uwis, N.J. (1977). Pesticides induced DNA damage and its repair in cultured human cells. Mutat. Res., 42: 161-174.

Al.Najjar, N.R. and Soliman, A.S. (1980). Cytological effects of fungicides. 1. Mitotic effects of Vitavax-200 and Dithane S-60 on wheat and two related species. Cytologia, 45 (1-2): 163-168.

Amer, S. (1965). Cytological effects of pesticides. I. Mitotic effects of N-methyl-1-naphthyl carbamate 'Sevin'. Cytologia, 30: 175-181.

Amer, S.M. and Ali, E.M. (1974). Cytological effects of pesticides. V. effects of some herbicides on Vicia faba. Cytologia, 39: 633-643.

Amer, S.M. and Ali, E.M. (1980). Cytological effects of pesticides XI. Meiotic effects of the herbicides monochloroacetic and trichloroacetic acids. Cytologia, 45: 715-719.

Amer, S.M. and Farah, O.R. (1968). Cytological effects of pesticides. III. Meiotic effects of N-methyl-1-naphthyl Carbamate 'Sevin'. Cytologia, 33:337-344.

Amer, S.M. and Farah, O.R. (1974a). Cytological effects of pesticides. VI. Effect of the insecticide 'Rogor' on the mitosis of Vicia faba and Gossypium barbadense. Cytologia, 39: 507-514.

Amer, S.M. and Farah, O.R. (1974b). Cytological effects of pesticides. VII. Mitotic effects of isopropyl-N-phenyl carbamate and 'Duphar'. Cytologia, 40: 21-29.

Amer, S.M. and Farah, O.R. (1976). Cytological effects of pesticides. VIII. Effects of the carbamate pesticides 'IPC', 'Rogor' and 'Duphar' on Vicia faba. Cytologia, 41: 597-606.

Amer, S.M. and Farah, O.R. (1979). Cytological effects of pesticides. IX. Effects of the phosphonothioate insecticide Leptophos on Vicia faba. Cytologia, 44: 907-913.

Amer, S.M. and Farah, O.R. (1980). Cytological effects of pesticides X. Meiotic effects of 'Phosvel'. Cytologia, 45: 241-245.

Bakale, V.L., Deshmukh, S.B. and Deshmukh, V.R. (1981). Effects of 2, 4-D on the cytology of Malvastrum coromandelianum (L). Garcke. In Perspectives in Cytology and Genetics (Eds.) Manna, G.K. and Sinha, U., Hindasia publishers, Delhi, 3: 289-293.

Bakale, V.L. and Hadke, S.M. (1981). Effects of herbicides 2,4-D, Sodium arsenite and Lasso on mitosis in Euphorbia geniculata Orteg. In Perspectives in Cytology and Genetics, (Eds.) Manna, G.K. and Sinha, U. Hindasia publishers, Delhi, 3: 295-298.

Bakale, V.L. and Kolhe, R.L.(1981). Mitotic abnormalities induced by herbicides in Solanum xanthocarpum, Schrad. and Wendl. In Perspectives in Cytology and Genetics, (Eds.) Manna, G.K. and Sinha, U. Hindasia publishers, Delhi, 3: 299-302.

Banerjee, R., Giri, A.K., Sharma, A. and Talukder, G.(1981). Chromosomal and cellular changes induced by organochlorine and carbaryl compounds. In Perspectives in Cytology and Genetics, (Eds.), Manna, G.K. and Sinha, U., Hindasia Publishers, Delhi, 3: 403-406.

*Barthelmess, A. (1957). Chemisch indzierete multipolare. Mitosenentwicklung. Amer. J. Bot.,54: 945.

Behera, B.N., Sahu, R.K. and Sharma, C.B.S.R. (1982). Cytogenetic hazards from agricultural chemicals 4. Sequential screening in the barley progeny test for cytogenetic activity of some systemic fungicides and a metabolite. Toxicol. letters, 10: 195-202.

Benes, V., Sram, R. and Tuscany, R. (1973). Testing of mutagenicity of fenitrothion. Mutat. Res.,21: 23-24.

Bhargava, Y.R. and Khalatkar, A.S. (1981). 2, 4-Dichlorophenoxy acetic acid as an environmental mutagen. Sixth annual conference, Environmental Mutagen Society of India, Calcutta. Jan. 27th to 29th 1981 (Abstract).

Bhojvaid, P.P. (1980). Some genetic and cytological effects of certain pesticides in laboratory mice (Mus musculus). M.Sc. Thesis submitted to the Haryana Agricultural University, Hissar.

Bhojvaid, P.P. and Vijayakumar, N.K. (1981). Cytological effects of endosulfan and carbaryl in laboratory mice (Mus musculus). Sixth annual conference of Environmental Mutagenic Society of India, Jan.27th to 29th 1981 (Abstract).

Blevins, R.D. (1977). Mutagenicity screening of five methyl carbamate insecticides and their nitroso acid derivatives using mutants of Salmonella typhimurium ht-. Mutat. Res.,45: 1-8.

Blevins, R.D., Lijinsky, W. and Regan, J.D. (1977). Nitrosated methyl carbamate insecticides effect on the DNA of human cell. Mutat. Res.,44: 1-7.

Bridges, B.A., Mottershead, R.P., Green, M.H.L. and Gray, W.J.H. (1973). Mutagenicity of dichlorvos and methyl methane sulphonate for Escherichia coli WP_2 and some derivatives deficient in DNA-repair. Mutat. Res., 19: 295-303.

Carere, A., Ortali, V.A., Cardamone, G., Torracca, A.M. and Rasbhetti, R. (1978). Microbiological mutagenicity studies of pesticides in vitro. Mutat. Res., 52: 277-286.

Carson, R. (1962). Silent Spring. Penguin Books, England. pp. 317.

Chen, H.H., Hsuch, J.L., Sirianni, S.R. and Huang, C.C. (1981). Induction of sister chromatic exchanges and cell cycle delay in cultured mammalian cells treated with eight organophosphorus pesticides. Mutat. Res., 88 (3): 307-316.

Cheng, M. and Conner, M.K. (1982). Comparison of sister chromatid exchange induction and known carcinogenic activities of venyl and allyl carbamates. Cancer Res., 42 (6): 2165-2167.

Council of the Environmental Mutagen Society (1975). Environmental mutagenic hazards. Science, 187: 503.

Czeizel, A., VanBao, T., Szabo, I. and Ruzieska, P. (1973). Human chromosome aberrations in acute organic phosphorous acid ester (pesticide) intoxication. Mutat. Res., 21: 187-188.

Darlington, C.D. and La Cour, L.F. (1976). The Handling of chromosomes. George Allen and Unwin Ltd. London, 6th Ed. pp.201.

*Darell, E., Griffin III, and Walter, E.H. (1978). In vitro breakage of plazmid DNA by mutagens and pesticides. Mutat. Res., 52: 161-169.

Datta, N. (1966). Cytological effects of gammexane on somatic chromosomes of Urginea coromandeliana, Hook. F. Curr. Sci., 35: 945.

*Dean, B.J. and Thorpe, E. (1972). Cytogenetic studies with dichlorvos in mice and chinese hamster. Arch. Toxicol., 30: 30-40.

Degraeve, N., Moustachen-Dahmen, M., Gilot-Delhalle, J., Colizzi, A., Houbrects, N. and Cholett, M.C. (1979). Genetic effects of organophosphate insecticides in mouse, Mutat. Res., 64: 131.

Deysson, G. (1968). Antimitotic substances. Int. Rev. cytol., 24: 99-148.

Dhingra, G. and Vijayakumar, N.K. (1981). Studies on the toxic and mutagenic effects of some pesticides in Drosophila melanogaster. Sixth annual Conference of Environmental Mutagen Society of India, Jan. 27th to 29th 1981 (Abstract).

*Dikstith, T.S.S. (1973). In vivo effect of parathion in chromosome of pig. Environ. Physiol. Biochem., 3: 161-168.

*Epstein, E. (1972). Detection of chemical mutogens by dominant lethal assay in mouse. Toxicol. Appl. Pharmacol., 23: 288-325.

Fahrig, R. (1974). Mutagenicity studies with pesticides in chemical carcinogen assays. IARC Scientific Publications, No.10.

*Fallon, R.D. and Fleirmans, C.B. (1980). Formation of nonvolatile mutagens by water chlorinations persistance and relationship to molecular weight of organic material in water. Chemosphere, 9 (7-8): 385-391.

*Fernandez-Gomez, M.E. (1967). Alteraciones en el ciclo de division cellular inducidas por los isomeros del HCCH. I. Isomeros alfa, beta, gamma. Genet. Iber., 19: 103-121.

Georgian, L. (1975). The comparative cytogenetic effects of aldrin and phosphamidon. Mutat. Res., 31:103-108.

*Georgieva, V. and Tsoneva, M. (1981). Sister chromatid exchanges in persons professonally exposed to Venyl chloride and polyvenylchloride. Genet. Sel., 14 (2): 132-139.

Giri, A.K., Banerjee, R., Talukdar, G. and Sharma, A.(1978). Chromosomal and cellular damages induced by organosphosphorus insecticides. Paper presented in Third All India Congress of Cytology and Genetics held at H.A.U., Hissar, on Oct. 23rd - 27th, 1978 (Abstract).

Giri, A.K., Banerjee, R., Talukder, G. and Sharma, A.(1981). Chromosomal and cellular changes induced by organophosphorus insecticides. In Perspectives in Cytology and Genetics, (Eds.) Manna, G.K. and Sinha, U. Hindasia Publishers, Delhi, 3:403-406.

Gopalaswamy, U.V. and Aiyar, A.S. (1981). DNA binding ability of lindane and hexachlorobenzene. Sixth annual Conference of Environmental Mutagen Society of India, Jan.27-29th, 1981 (Abstract).

Gonzalez-Fernandez, A., Lopez-Saez, J.F., Gimenez-Martin, G. (1966). Duration of the division cycle in binucleate and mononucleate cells. Exp.Cell Res., 43: 255-267.

Grant, W.F. (1978). Chromosome aberrations in plants as a monitoring system. Environ. Health Perspect., 27: 37-43.

Grant, W.F. (1982 a). Cytogenetic studies of agricultural chemicals in plants. In Genetic Toxicology: An agricultural perspective. (Eds.) R.A.Fleck and A. Hollaender. Plenum Press, New York.pp.353-378.

Grant, W.F. (1982 b). Chromosome aberration assays in Allium. A report of the U.S. Environmental protection Agency Gene-Tox Program. Mutat. Res., 99: 273-291.

Hanna, P.J., and Dyer, K.P. (1975). Mutagenicity of Organophosphorous compounds in bacteria and Drosophila. Mutat. Res., 28: 405-420.

*Herichova, A. (1970). Study of the effect of isopropyl-N-carbamate and isopropyl-N-(3-chlorophenyl) carbamate on chromosome structure and cytokinesis. Acta. F.R.N. Univ. Comen. Physiol. Plant., 1: 147-154.

Jagoda, M. (1980). Cytological disturbances in Allium cepa, L. root meristems induced by herbicides. Acta.Biol. Cracova, 22: 189-211.

Johnson, C.A. and Jalal, S.M. (1973). DDT induced chromosomal damage in mouse. J. Hered., 64 (1): 7-8.

Kar, S. and Singh, P.K. (1980). Mutagenicity of pesticides carbofuran and hexachlorocyclohexane to blue-green alga, Nostoc musocorum. Microbios. Lett., 12(46): 79-82.

Kihlman, B.A. (1966). Actions of chemicals on dividing cells. Englewood Cliffs, N.J., Prentice Hall, New Jersey, pp. 260.

Kiraly,J., Cseizel, A. and Szentesi, I. (1977). Genetic study on workers producing organophosphate insecticides. Mutat. Res., 46: 224.

*Klasterska, I., Natarajan, A.T. and Ramel, C. (1976). An interpretation of the origin of subchromatid aberrations and chromosome stickiness as a category of chromid aberrations. Hereditas, 83: 153-162.

*Komala, Z. (1978). The effect of lindane on Paramecium primaurelia. Folia Biol. (Cracow), 26(4): 355-360.

*Kostoff, D. (1931). Hiteroploidy in Nicotiana tabacum and Solanum melongena caused by fumigation with nictine sulfate. Bull. Soc. Bot.Bulgar., 4: 87-92.

Kramers, P.G.N. and Knaap, A.G.A.C. (1975). Mutagenicity tests with captan and folpet in Drosophila melanogaster, Mutat. Res., 21: 149-154.

Kramers, P.G.N. and Knaap, A.G.A.C. (1978). Absence of a mutagenic effect after feeding dichlorvos to larvae of Drosophila melanogaster. Mutat. Res., 52: 103-105.

*Kucerova, M., Polivkova, Z., Saram, R. and Matousek, V. (1976). Mutagenic effect of epichlorhydrin. Testing on human lympocytes in vitro in comparison with TEPA. Mutat. Res., 34: 271-278.

*Leoni, V., D'Alessandro de Luca, E., Belisario, M.A. and Pampena, A.(1982). On the N-nitroso derivates of three pesticides (atrasine, carbaryl, chlorobromuron) used in agriculture. Ig. Mod., 78(3): 301-320.

Lessa, J.M.M., Becak, W., Nazarethrabello, M., Pereira, C.A.B. and Ungaro, M.T. (1976). Cytogenetic study of DDT on human lympocytes in vitro. Mutat. Res., 40: 131-138.

Lofroth, G., Kim, C. and Hussain, S. (1969). Alkylating property of 2, 2-dichlorovinyl dimethyl phosphate: A disregarded hazard. Environmental Mutagen Society, News letter, 2: 21-26.

Levan, A. (1938). The effect of colchicine on Root Mitosis of Allium. Heriditas, 24: 471-486.

*Logvinenko, V.F. and Morgan, V.V.(1978). Study of mutagenic effects of some pesticides on durum spring wheat. Tsitol. Genet., 12(3): 207-212.

*Logvinenko, V.F. and Morgan, V.V. (1982). A study of mutagenic pesticide activity in higher plants. Tsitol. Genet., 16 (3): 63-72.

Mahu, U. and Herbet, G.M. (1975). The effect of insecticides on Chinese hamster cell cultures. Mutat. Res., 40: 107-118.

Markaryan, D.S. (1967). Effect of dieldrin on the mitosis in Crepis capillaris sprouts, Genetika, 3: 55-58.

Marucka, S. and Yamanka, S. (1980). Production of mutagenic substances by chlorination of water. Mutat. Res., 79 (4): 381-386.

McCoy, E.C., Burrows, L. and Rosenkraz, H.S.(1978).Genetic activity of allyl chloride, Mutat. Res., 57: 389-392.

*Mensinkai, S.W.(1939). J.Genet., 39: 1-45. (c.f.) Grant, W.F. (1982b). Chromosome aberration assays in Allium. A report to the U.S. Environmental Protection Agency Gene-Tox Program, Mutat. Res., 99: 273-291.

Mercykutty, V.C. and Stephan, T. (1980). Adriamycin induced genetic toxicity as demonstrated by the Allium test. Cytologia, 45: 769-777.

Mishra, G.M. and Sinha, S.P. (1979). Effects of malathion on mitotically dividing onion (Allium cepa) root tip cells. Indian J. Exp. Biol., 17: 716-717.

Mishra, G.M., and Sinha, S.P. (1981). Effects of Malathion on mitotic index in Onion root tip cells. In Perspectives in Cytology and Genetics, 3: 479-482. Hindasia Publishers, Delhi.

Mohn, G. (1973). Comparison of the mutagenic activity of 8 organophosphorous insecticides in Escherichia coli. Mutat. Res., 21: 196.

Mohandas, T. and Grant, W.F. (1972). Cytogenetic effects of 2, 4-D and Amitrole in relation to nuclear volume and DNA content in some higher plants. Can. J. Genet. Cytol., 14: 773-783.

Morrison, J.W. (1962). Cytological effects of the herbicide 'Avadax'. Can. J. Pl. Sci., 42: 78-81.

Nicholas, A.H., Vienne, M. and Berghie, H. VanDen (1979). Induction of sister chromatid exchanges in cultured human cells by an organophosphorous insecticide - malathion. Mutat. Res., 67: 167-172.

Nazarethrabello, M., Becak, W., Dealmeida, W.F., Pigati, P., Ungaro, M.T., Murata, T. and Pereira, C.A.B.(1975). Cytogenetic study on individuals occupationally exposed to DDT. Mutat. Res., 28: 449-454.

Pal, B.C., Cumming, R.B., Walton, M.F.and Preston, R.J.(1981). Environmental pollutants. 5-chlorouracil is incorporated in mouse liver and testis DNA.Mutat. Res., 91(4): 395-401.

Panda, B.B. and Sharma, C.B.S.R. (1980). Cytogenetic hazards from agricultural chemicals 3. Monitoring the cytogenetic activity of trichlorfon and dichlorvos in Hordeum vulgare. Mutat. Res., 78: 341-345.

Parry, J.M. (1973). The induction of gene conversion in yeast by herbicide preparations. Mutat. Res., 21: 83-91.

Picciano, D. (1978). Cytogenetic investigation of occupational exposure to epichlorhydrin. Mutat. Res., 66: 169-173.

Prasad, I and Pramer, D. (1968). Genetic effects of ferbam on Aspergillus niger, and Allium cepa. Phytopathol., 58: 1182-1189.

Reddy, M.V. and Rao, B.V.R. (1969). The cytological effects of insecticides (Dimecron-100 and Rogar-40) on Vicia faba, L. Cytologia, 34: 408-417.

Sahu, R.K., Behera, B.N. and Sharma, C.B.S.R. (1981). Effects of a fungicide, dexon and its derivative on root meristems. The Nucleus, 24 (1,2): 60-65.

Sarbhoy, R.K. (1980). Effect of paradichlorobenzene on the somatic chromosomes and mitosis of Lens esculenta (L). Moench. Cytologia, 45 (3): 381-388.

Scholes, M.E. (1955). The effects of aldrin, dieldrin, isodrin, endrin and DDT on mitosis in roots of onion (Allium cepa, L.). J. Hort. Sci., 30: 181.

Sharma, C.B.S.R., and Sahu, R.K. (1977). Cytogenetic hazards from agricultural chemicals. 1. A.preliminary study on the responses of root meristems to exotoxin from Bacillus thuringiensis a constituent of a microbial insecticide, thuricide. Mutat. Res., 46: 19-26.

Sharma, Y.P. and Agarwal, K. (1980). Cytomorphological studies on para-dichlorobenzene treated maize. Sci. Cult., 46 (4): 153-155.

*Shaw, A. and Garner, R.C. (1983). The biological activity of 4-chloromethyl biphenyl, benzyl-chloride and 4-hydroxymethyl biphenyl in 4 short term tests for carcinogenicity. A report of an individual study in the UKEMS genotoxicy trial 19, Mutat. Res., 119 (2): 121-133.

*Shelby, M.D. (1976). Chemical mutagenesis in Plants and mutagenicity of plant related compounds. ORNL/EMIC-7. Oak Ridge, Tenn: Oak Ridge National Laboratory.

Shirasu, Y., Moriya, M. Kato, K., Furuhashi, A. and Kada, T. (1976). Mutagenicity screening of pesticides in the microbial system. Mutat. Res., 40: 19-30.

*Shokod'ko, T.I., Merezhko, A.I. and Lyashenko, A.N.(1978). Effects of DDT and BHC on animilation and outflow of ^{14}C in Phragmites communis. Gidro. Biol. ZL., 14 (4): 105-109.

Siebert, D. and Eisenbrand, G. (1974). Induction of mitotic gene conversion in Saccharomyces Cerevisae by N-nitrosated pesticides. Mutat. Res., 22: 121-126.

Singh, B.D., Singh, Y., Singh, R.B., Singh, R.M., Sarma, N.D.R.K. and Singh, J. (1977). Cytogenetic aberrations and morphological changes induced by insecticide treatments of barley seeds. Indian J. Exp. Biol., 15: 688-691.

Smith, M.J.A., Trevino, J. and Ring, R. (1972). Mutagenicity of dichlorvos, Nature, 240: 418-420.

Sobels, F.H. and Todd, N.K. (1979). Absence of a mutagenic effect of dichlorvos in Drosophila melanogaster. Mutat. Res., 67: 89-92.

*Sobti, R.C., Krishan, A. and Pfaffenberger, C.D. (1982). Cytokinetic and cytogenetic effects of some agricultural chemicals on human lympoid cells in vitro : Organophosphates. Mutat. Res., 102(1): 89-102.

Soliman, A.S. and Al-Najjar, N.R. (1980). Cytological effects of fungicides. II. Chromosomal aberrations induced by Vitavax-200 and Dithane S-60 in meiotic cells of wheat and two related species. Cytologia, 45(1-2): 169-175.

Srivastava, L.M. (1966). Induction of mitotic abnormalities in certain genera of Tribe-Viciae by para-dichlorobenzene. Cytologia, 31: 166-171.

Srivastava, S., and Sarma, Y.S.R.K. (1979). Effect of two insecticides, dimecron and nuvan, on the survival, growth and nuclear cytology of Oidogonium quinii, Wittr. J. Cytol. Genet., 14(2): 163-172.

Stephan, J. (1979). Cytological causes of spontaneous fruit abortion in Haemanthus katharinae, Baker. Cytologia, 44: 805-812.

Sterrett, R.B. and Fretz, T.A. (1975). Asulam induced mitotic irregularities in onion root tips. Hort. Sci., 10: 161.

*Storey, W.B. and Mann, J.D. (1967). Chromosome construction by O-isopropyl-N-phenyl carbamate, IPC, in Vicia faba and Cycas circinalis. Stain Tech., 42(1): 15.

Storey, W.B., Jordan, L.S. and Mann, J.D. (1968). Carbamate herbicides - Newtools for cytological studies. Califorina Agriculture , 22: 12-13.

Subhash, K. and Rajam, M.V. (1983). Cytological and morphological variations induced in Capsicum by X-irradiation. Indian J. Bot., 6 (1): 29-32.

*Tanaka, N. (1956). Spontaneous chromosome aberrations in the root meristem of Tradescantia paludosa. La Kromosome, 29: 1010-1019.

Tomkins, D.J. and Grant, W.F. (1976). Monitoring natural vegetation for herbicide induced chromosomal aberrations. Mutat. Res.,36: 73-84.

*VanBao, T., Szabo, I., Ruzieska, P. and Czeizel, A. (1974). Chromosome aberrations in patients suffering from acute organic phosphate insecticide intoxication. Humangen, 24: 33-57.

Vasudev, V. and Krishnamurthy, N.B. (1981). Toxicity and mutagenicity studies with baygon in Drosophila melanogaster. Sixth annual conference Environmental Mutagen Society of India, Jan.27-29th 1981 (Abstract).

Vijayakumar, N.K., Dhingra, G. and Bhojvaid, P.P.(1981). Epichlorhydrin - A potent genotoxic compound. Sixth annual conference of Environmental Mutagen Society of India, Jan.27-29th 1981 (Abstract).

Wild, D. (1975). Mutagenicity studies on organophosphorous insecticides. Mutat. Res., 32: 133-150.

Worthing, C.R. (1979). The pesticide manual, a world compendium. Published by the British Crop Protection Council, 6th ed. pp.655.

Wuu, K.D. and Grant, W.F. (1966). Induced abnormal meiotic behaviour in a barley plant (Hordeum vulgare, L.) with the herbicide Lorox. Phyton, 23 (1): 63-67.

Wuu, K.D. and Grant, W.F. (1967). Chromosomal aberrations induced by pesticides in meiotic cells of barley. Cytologia, 32: 31-41.

Yoder, J., Watson, M. and Benson, W.W. (1973). Lympocyte chromosome analysis of agricultural workers during extensive occupational exposure to pesticides. Mutat. Res., 21: 335-340.

* Originals not seen

CYTOTOXIC AND CLASTOGENIC EFFECTS OF SOME INSECTICIDES IN *Allium cepa* L.

By

JAYAPRAKASH NAIK, B

THESIS

Submitted in partial fulfilment of
the requirement for the Degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University

Department of Agricultural Botany
COLLEGE OF HORTICULTURE
Vellanikkara, Trichur - 680 654
KERALA INDIA

1983

ABSTRACT

In the present investigation, the cytotoxic and clastogenic effects of three very commonly used insecticides, namely, aldrin, carbofuran and phorate were tested in Allium cepa, L., a test system.

Four concentrations of each insecticides were used for the study. These concentrations were fixed taking into consideration of their field dose of application in insect control. The treatment periods fixed were 12, 24 and 48 hours. The mitotic index was computed from 4000 cells and indices of each division phases were also computed. The chromosome abnormalities were scored from about 100 to 150 cells of each phases in each treatments.

Aldrin was found to be drastically mitodepressive compared to carbofuran and phorate. While phorate increased mitotic index in the lowest dose, carbofuran showed only marginal reduction. However, both the compounds reduced mitotic index in higher doses and the field doses. Aldrin exhibited a dose and period responsiveness, while carbofuran and phorate could not with regard to mitotic index.

The study also revealed that aldrin is an effective toxicant on both genetic material and proteins. The various chromosome abnormalities noticed were stickiness, bridges, laggards, blurred chromosome borders, chromatin

bridge, micronuclei, non-orientation of metaphase, precocious movement in anaphase, chromatin bodies, irregular anaphase, star metaphase, strays, beaked nuclei, break, C-metaphase and unequal nuclei in their decreasing order of occurrence. Unlike aldrin, carbofuran and phorate could not induce anomalies to a significant level in the lowest doses tried, 0.0075 and 0.02 per cent respectively. Chromosome bridge being the most frequent abnormality found in carbofuran, which was followed by stickiness and laggards. The frequency of abnormalities found in field and higher doses were more or less same. Phorate, on the other hand showed linear relationship in inducing chromotoxicities with respect to concentrations and period of treatment. The major types of abnormalities recorded were bridges, breaks and stickiness.

The results showed that all the insecticides tried were capable of affecting the genetic material as well as protein, but to different degrees depending on concentrations. It can be tentatively concluded that they cannot be considered completely safe at the field dose of application on the cellular constituents of the organism. The results call for extensive testing of these chemicals in other test systems also.