CYTOTOXIC AND BIOCHEMICAL EFFECTS OF SOME ORGANOPHOSPHORUS PESTICIDES IN *ALLIUM CEPA* L. AND *ALLIUM SATIVUM* L.

Thesis

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By

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CERTIFICATE

This is to certify that the thesis entitled "Cytotoxic and biochemical effects of some organophosphorus pesticides in *Allium cepa* L. and *Allium sativum* L." is an authentic record of work carried out by Sr. Kochuthressia M.V. during 2001 – 2005 under my supervision and guidance and that no part thereof has been presented earlier for any other degree or diploma.

Dr. John E. Thoppil

DECLARATION

I here by declare that the thesis entitled "Cytotoxic and biochemical effects of some organophosphorus pesticides in *Allium cepa* L. and *Allium sativum* L." submitted for the degree of Doctor of philosophy in Botany of Calicut University, is a research work done by me under the guidance of Dr. John E. Thoppil, Reader, Genetics and Plant Breeding Division, Department of Botany. This has not been submitted earlier for any other degree or diploma.

Calicut University

30- 06 -2005

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INTRODUCTION

Indian agriculture is witnessing a new era of changes and opportunities as we enter the new millennium. The food grain production had increased from 51 million tons during 1950 to 209 million tons during 2000. In the same period, there was a three-fold increase in population. The increase in grain production was mainly from the wheat and rice in the green revolution era.

In the developing countries like India, agriculture sector has been a principal source of employment and income. Moreover it provides raw materials to industry and forms much of export. Agriculture in India is a major source of economy. It contributes 50% of the total national income and gives direct employment to about 68% of the total population and nearly 90 % of the population of rural area and provides nearly 35% of the country's export. Hence it is not surprising that agriculture in India has been receiving much attention in the sectoral allocation of inputs in the Five Year Plans in India and therefore top priority has been assigned to develop agriculture sector in our country.

One of the main problems India had faced was the scarcity of food for the increasing population. As the existing agricultural land was not enough for cultivation, India had to strive for the increase in production on the existing land. In the last four decades, India gained more than triple of its food production.

Urbanization of cultivable land is a pathetic truth, which we are facing in these days. Man's way of thinking and style of life gave way for the weakness of agriculture sector. What we think now is how to make better production from the available agricultural land. The cultivation of high yielding varieties of crops has amply demonstrated that their high yield potential can be realized only by adequate plant protection measures.

Pest infestation is what affects our agriculture a lot. In India, crop failure due to pest infestation often led to severe famine like the Bengal famine of 1942. Infestation by *Helminthosporium* affected the rice crops then.

Man started his fight against the pest centuries before. The middle of the 20th century is a revolutionary time in the field of pest control. During the World War II, the insecticide properties of DDT were discovered and this synthetic organic substance was successfully utilized against the pests. After the war chemical researchers brought out an array of pesticides. Pesticides showing many advantages were commonly accepted all over the world. The benefit that the insecticide revolution has brought to mankind stands as a remarkable testimonial to human intelligence and technological prowess. The benefits measured in terms of lives served, diminished suffering and economic gains are inestimable (Metcalf, 1965).

There can be no doubt that from the standpoint of human welfare, the insecticide revolution has been an enormously fortunate event. However, problems have arisen, some quite serious, to detract from the benefit realized out of the new insecticides (Bartlett, 1964; Rudd, 1964). Pest control is largely an ecological matter. Consequently modern insecticides have, with distressing frequency, engendered serious problems, through their disruptive impact on the ecosystems to which they have been applied.

More than 68,000 species of insects among the approximately 8,00,000 are considered as pests (Walter *et al.*, 1980). In specialized agriculture, the wide distribution of plant diseases to cereals, fruit crops, cotton, sugar beets, legumes, vegetables, potatoes and grapevines necessitate annual large-scale repeated chemical treatments against complexes of insect pests and disease causing agents, which damage these crops. Every year a number of new pesticides are being introduced in agriculture to overcome the problems of pests and diseases.

The pesticides have also played a very important part in raising the productivity of labour in agriculture. The investigations are still continuing because agriculture is an important profession in developing countries like India

and hence it is inevitable that it should be tuned to the changing needs of the society. Modern agricultural practices were adopted so as to keep pace with the alarmingly increasing incidence of pests, diseases as well as weeds and also the increase in human population.

The word "pesticide" has been gradually adopted as a collective term to describe a wide range of chemicals now used to manipulate biological systems, in agriculture and other industries. The "Review of Present Safety Arrangements for the use of Toxic Chemicals in Agriculture and Food Storage" (Cook, 1967) defines a "pesticide product" as including chemicals used to destroy any insect, fungus, bacterium, virus or rodent or to attract, repel, sterilize, any pest, or to act as a plant growth regulator, defoliant and desiccant. For practical purposes, many chemicals with industrial uses and virtually all-agricultural chemicals, with the exception of inorganic fertilizers, may be include within the definition and can be considered as pesticides. Their injudicious use leads to environmental pollution. In recent years, the uses of chemical pesticides have been increasing. This has led to an intensive search for alternative strategies of plant protection that can be used safely to control pests. The consumption of pesticide is concentrated more on irrigated crops. It has been clearly indicated by several workers that widespread use of pesticides has resulted in the imbalance of the natural biological system (Wain Wright, 1978). The problem of residue accumulation was realized two decades ago. The undesirable environmental effects of pesticides and industrial pollutants were also reported. These effects include excessive mortality and reduced reproductive potential in birds, fishes and other organisms. Changes in the abundance of species and variation in the diversity of ecosystem, a reduction in the productive potential of natural resources and the development of pesticide-resistance also occurred.

The number of agricultural chemicals used for the protection of crops against pests has multiplied in the past two decades. It has become clear that many of these chemicals have properties similar to the chemicals and radiation used in mutagenesis. Chemicals with mutagenic action will cause environmental hazards and tests need to be undertaken to determine which among the more than 400 registered pesticides can be safely used for crop protection. The uses of pesticides are unavoidable in modern agriculture. Several million tons of organic and inorganic chemicals with antimicrobial and insecticidal properties are added annually to our environment. Some of them while killing or inhibiting the harmful organisms not only upset the ecosystem but also produce undesirable changes in higher organisms (Jain and Sarbhoy, 1988).

The recent developments in agro-technology have accelerated the use of pesticides to a great extent. The danger from the wide scale use of pesticides lie in the possibility that it may damage the hereditary material of man. Advisory panel on mutagenicity of pesticides to the Secretary's Commission to environmental health has recommended that each and every chemical being used as a pesticide be scanned for its mutagenicity (Lederberg, 1971). Thus the responsibility for safety testing against mutagenicity falls in the hands of disinterested third parties. Grover and Tyagi (1979) found that at present more than 400 chemicals are being used as pesticides.

The human population encounters enormous and diverse kinds of chemicals like food additives, cosmetics, drugs, pesticides, household materials and occupational substances. It has been estimated that about half a million different chemicals are in use through out the world and approximately 10,000 of them are produced annually in quantities between 500 and 1,00,000 kg. (UNEP, 1975). The screening for mutagenicity of environmental chemicals have become a major enterprise in biosciences in many parts of the world due to subtle danger

of them being mutagenic and may cause mutations of somatic cells resulting in the formation of malignant cells in the individuals.

The plant growth characters are controlled by cell division, which was affected by treatment with new chemicals of agriculture. Therefore, many investigators have tried to demonstrate the effect of these chemicals on mitosis and meiosis (Mousa, 1982). All these chemicals affected the rate of mitotic division and produced different kinds of chromosomal abnormalities.

Several bioassays have been proposed for the monitoring of mutagenicity of environmental chemicals (Kilbey *et al.*, 1977). The chromosome aberration assay in barley (*Hordeum vulgare*) is being recommended as one of the bioassays in gene-tox programmes.

Pesticides (insecticides, herbicides and fungicides) play a very important role in protecting vegetables, fruits and food grains right from the seed stage through germination to growth and then to storing. However, their use has many undesirable effects. Brown (1962-63) studied many environmental mutagens; the conclusion has been reached that they can induce considerable damage into the human gene pool (Grant, 1971). The majority of mutation induced by them may be recessive and of little significance to the individual carrying them but when brought together at the time of conception, they may produce harmful effects to the developing embryo (Grant, 1970). Thus constant use of these pesticides may result in changing the hereditary constitution of the organism (Wuu and Grant, 1966a, 1967a).

Increased utilization of pesticides for crop protection in modern agriculture has raised the question whether these chemicals induce any detectable cytological damage to the cells. Though the use of pesticide has become a necessity, the frequent and indiscriminate use of these chemicals proved to have many undesirable secondary consequences on higher plants (Amer and Farah,

1974). Many workers reported the mutagenic effects of a wide range of insecticides from time to time (Fugii and Tadashi, 1983; Gavirila, 1984).

Plant based residues of most of these agrochemicals have been reported to be genotoxic (Stevens, 1971; Sinha, 1988). A number of workers have carried out studies to show the cytological effects of different agro chemicals on different plant species (Kumar and Sinha, 1989).

Although the plant protection chemicals are potentially hazardous in the human environment, their use has been markedly increased during the last few years. While the use of these chemicals may have many undesirable secondary consequences (Imbamba and Moss, 1971), their repeated use may even induce insecticide resistance in pests (Keer, 1963). Chromosomal damages have been regarded as one of the dependable parameters for genetic injury and serve as an indicator of environmental mutagenesis. It is quite apparent from the literature and finely a large number of pesticides have been proved to be effective mutagenic agents and demonstrated to induce chromosomal alterations in mitotic (Wuu and Grant 1966b; Somasekhar *et al.* 1984) system of various plants. In view of the importance of the problem, before the use of insecticides to control pests, it is better to understand the cytogenetic response of the crop under study for safe and effective use.

Evidences accumulated in the past two decades have indicated that a countless number of pesticides are capable of inducing chromosomal damages in crop plants and gave a review of the various types of chromosomal anomalies and the pesticides that produce them. Moreover a link between chromosomal anomalies produced by these compounds and gene mutation has been confirmed (Panda and Sharma, 1989). The use of chromosomal aberrations induced by pesticides in crop plants, is therefore being accepted as indicators of genetic damage (Ma, 1982). The use of plant root tips particularly those of *Allium cepa*

and *Vicia faba* as a bioassay test system for the genotoxicity of pesticides has shown extremely good correlation with the bacterial and mammalian systems (de Kergommeaux *et al.*, 1983).

Another common effect of pesticide is the inhibition of cell division. Some workers have attributed the inhibition of cell division by these chemicals to an action on DNA and RNA in the cells (Tomkins and Grant, 1972; Yoshida *et al.*, 1983). Chromosomal damage produced by pesticides may also involve an action on DNA.

According to Brusick (1982) many hereditary diseases found in human beings might have arisen due to exposure to harmful chemicals and radiations. Cancer epidemiologists claimed that at least 80 % of cancers were caused by environmental agents especially carcinogenic chemicals and radiations (Ames and Mc Can, 1976).

Levan (1949) introduced the '*Allium* test' for screening chemicals for clastogenic activity and Kihlman (1956) introduced the *Vicia faba* test system for the same purpose. However only after the development of 'Ame's test' (Ames, 1971) and its worldwide acceptance in the screening of chemicals for mutagenic activity, enthusiastic scientists began to work hard to develop additional short-term test systems. A number of such short term assays so developed gained international recognition when the International Agency for Research on Cancer (IARC) recommended these tests (Montesano *et al.*, 1976) in 'Genetic Toxicology Testing'.

Quite a lot of work has already been done under the auspicious of the Environmental Protection Agency of United States, International Commission for Protection against Environmental Mutagens and Carcinogens, Netherlands and several other laboratories around the world screened the various chemicals to which human beings are exposed for the evaluation of their toxicity in terms of

risk to human health and heredity. IARC has already published several monographs on the screening of chemicals for mutagenic and carcinogenic properties. Works on several therapeutic drugs, food additives, water pollutants, pesticides, artificial manures and industrial effluents are in progress and several research papers have already been published (Abraham and Cherian, 1976).

Modern crop protection not only comprises the use of insecticide but also the introduction of fungicides and herbicides. There are four chemical types of insecticides. (1) Organochlorins (2) Organophosphates (3) Carbamates and (4) Pyrethroids. The organophosphate insecticides are less persistent than the organochlorins. The group of insecticides having phosphorous as the active nucleus are referred commonly as the "organophosphates". It is the largest and most diverse group of insecticides known. These insecticides are esters of alcohols with phosphoric acid or anhydrides of phosphoric acid with another acid. Thus they can be chemically reactive. They are the product of the research on "War Gas" during World War II. They act on the nervous system by inhibition of acetyl cholinesterase. In this, they share a common site with the carbamates.

Since the early 70's organophosphorus compounds are effective cholinesterase inhibitors in insects and have become the most extensively used class of insecticides. Most of these are esters or amide esters of phosphoric acid (Wild, 1975).

The organophosphate insecticides are chemically unstable, and have virtually replaced the persistent organochlorine compounds. This is especially true with regard to their use around the house and garden. They have several commonly used names. They are organic phosphates, phosphorus insecticides, nerve gas relatives, phosphates, phosphate insecticides and phosphorous esters or phosphoric acid esters. They are all derived from phosphoric acid and are

generally the most toxic of all pesticides to vertebrate animals. Because of their chemical structure and mode of action, they are related to the "nerve gases" Sarin, Soman, and Tabun. Their insecticide action was observed in Germany during World War II. This association of organophosphorus insecticides with war gas was hardly an auspicious beginning for a group of compounds, which was destined to become one of the main weapons in our armory against insect pests of importance in agriculture, public hygiene and medicine (Hassall, 1982).

Organophosphorus compounds are one of the most important groups of modern pesticides. The wide spread use of their compounds are due to (1) high insecticidal and acaricidal activity (2) broad spectrum (3) rapidity (high initial toxicity) of action on pests (4) low stability in biological media (5) decomposition with the formation of products nontoxic to men and animals (6) absence of an ability to be deposited inside the body of men, animal or plants (7) systemic action of a number of these compounds (8) low rate of use per unit area treated and (9) rapid decomposition in soil and water.

They have two distinct features. (1) They are generally much more toxic to vertebrates than the organochlorine insecticides (2) They are chemically unstable or non-persistent. The latter quality brings them on to the agricultural scene as substitutes for the persistent organochlorines.

Organophosphorus insecticides are widely used chemicals in modern agriculture and public health. A number of adverse reactions have occasionally been observed after routine treatment with various organophosphorus compounds in domestic animals and plants. Evaluation of the genotoxic effects of these chemicals is of great importance since their extensive use could be detrimental to the ecosystem in general and plants in particular. A considerable amount of attention has been focused on the organophosphorus compounds due to their impressive cytological and biochemical effects. Chemically induced

chromosomal anomalies have been used as a rapid indicator of potential mutagenic, carcinogenic and teratogenic activity of environmental mutagens. There has also been evidence for a correlation between chromosomal damage and toxic effects of chemical treatment. Thus plant and animal ecosystem are adversely affected by the application of pesticides. This underlines the need for extensive studies on the biochemical and cytological impact of pesticides. Cell division, nucleic acid metabolism and protein synthesis and amino acid metabolism are the primary field of action of pesticides, because these processes are essential for continued plant growth and development. When these agrochemicals interact with genetic material, causing DNA alterations in the germ cells, they may become harmful to the future generations.

The pesticide chemical may remain in or on the crop, or in the soil, drift to other nearby crop areas, flow into streams and drainages and there by create a hazard to man or his animals or produce additional side effects. These chemicals may create hazards to pollinators, wild life, and other beneficial forms. Finally product contamination or environmental pollution can lead to legal issues.

Any modification of the environment may have extensive ecological implication. An attempt to make the environment unfavourable to a particular pest organism may make it favourable to something else. The use of chemicals has dramatized this point in a most spectacular fashion and has also demonstrated that unilateral use of chemicals may end in disaster.

At this juncture, it is to understand how pesticides have affected man, and how man will continue to be affected in the future. Pesticides do exactly what man intends, that is to say they kill pests. They do so in the majority of cases without harming man himself and with minimal effect on man's environment. There have been serious incidents when human beings have endured harmful effects on living organisms other than they wish to exterminate. We need to

know whether in the future, pesticides will become more dangerous and do more damage to the environment, whether they will become safer or whether we will be able to decrease their use, or even to stop using them altogether. But the cases show that the correct and careful use of pesticides should always be accompanied by a proper scheme for the development of the country concerned.

Therefore man must learn to live with pesticide. He can cut down their use, by accepting a small amount of damage to his crops, many of which may be over-protected for cosmetic reasons. He can encourage researches to find safer and less-toxic pesticides and to find acceptable non-chemical methods of control. But he must accept the fact that he is using toxic substances to kill living organisms and that some harm may occur to man himself and to the environment. The present investigation aims to reveal the harmful effects of five commonly used organophosphorus pesticides using the 'Allium test'. The test materials used were *Allium cepa* and *Allium sativum.* The genus *Allium* belongs to the tribe Alliaceae of family Liliaceae (Bentham and Hooker, 1880). These *Alliums* are bulbous biennial or perennial herbs, which give off distinctive and pungent odour of onion or garlic when tissues are crushed. They are propagated from seeds, bulbs, cloves and bulbils (Jones and Mann, 1963). The unpleasant and characteristic odours from fresh onion and garlic sliced or crushed is due to the organic sulphur compound, allicin, which possess potent antibacterial properties (Cavallito *et al.*, 1944). A colourless, odourless water-soluble compound known as allein is present in uninjured garlic. On injury of the cells, an enzyme allicinase comes in contact with allein and causes its break down into sulphur containing product allicin (Abraham, 1976).

The onion (*Allium cepa*) is one of the most important commercial vegetable crops grown in India. It is grown in North as well as South India. The most important onion growing states are Maharashtra, Tamil Nadu, Andhra,

Bihar and Punjab. The characteristic flavour accounts for its popularity. Onion has been used as a vegetable from very ancient times.

The demand for *Allium* is worldwide. They are used primarily as a salad and cooked in various ways in all curries, fried, boiled, baked, used in soup making, in pickles and for other purposes. Germans call the onion "the Queen of the kitchen" because it plays such an important role in their cooking. Onion is rich in phosphorus, calcium and carbohydrates. It contains protein and vitamin C also. The flavour of onion is due to the presence of a volatile oil known as allyl propyl disulphide. The bulbs are used as food and as flavouring substance. The nutritive value of onion varies from variety to variety.

Scape of onion is 60-70 cm in height, ventricose, leaves sub-distichously fistular, acute, and shorter than the inflated scape. The flowers are greenish white, stellate, in large globular umbels with or without bulbils and enclosed by a thin membraneous spathe. Fruit is a capsule. The rudimentary stem is pungent, useful in vomiting, body pains, tumours, bleeding piles and epitasis. The bulb has a sharp taste, tonic, stomachic appetizer, useful in malaria, ophthalmia, diseases of the spleen, asthma, scabies, ear-ache, piles, enriches the blood of women, applied to the eyes in night-blindness (Govil, 1998).

Onion has been used medicinally for centuries as an external antiseptic. Studies conducted indicate that onion may be helpful in allaying intestinal gas pains, in reducing hypertensions, high blood sugar (as in diabetes) and the cholesterol and fat content of the blood, and relieving pain and inflammation. Both raw and cooked onion is a popular vegetable throughout the world (James and David, 1990).

Garlic is botanically known as *Allium sativum* Linn. (Family Liliaceae) and is one of the most commonly used spices with other vegetables and add flavour to the preparations. It is rich in protein, phosphorus, potash, calcium and

magnesium. It is rich in carbohydrates also. Besides, it contains fat, vitamin C and sulphur. The flavour of garlic is due to the presence of the chemical allein plus diallyl disulphide. Bulbs are used as a condiment and flavouring substance. Garlic powder is extensively used as a condiment and also serves as carminative and gastric stimulant. They also possess antiseptic and antibacterial properties. Garlic, the native of Siberia, is a gift to the earth and has immense medicinal properties. It is good for cough, indigestion, gastric disorders, cuts and wounds, pneumonia, bronchitis, tuberculosis and asthmatic complaints. To some extent both onion and garlic are antibiotic.

A. sativum is cultivated in India and many other parts of the world. Garlic occurs as a sub-globular, compound bulbs, grayish white, and 4 to 6 cms. in diameter with several (8-20) smaller bulbs called cloves. The whole surrounded by 3-5 whitish papery membraneous scales from the leaf bases of the previous year's bulb and terminating in a thick papery outgrowth. The cloves are attached to a flattened circular, woody axis with numerous thin, wiry roots on the underside and short, sub-cylindrical out growth on the upper surface.

Each clove is ovoid, 3-4 sided surrounded by two papery scale leaves, the outer one whitish and loose, the inner one pink and adherent, but easily separable from the solid portion of the clove. These papery scale leaves enclose two whitish, fleshy scales, the inner one thinner and smaller than the outer. The cultivation of garlic as commercial crop is similar to that of onion.

The production of garlic in India is increasing. It was about 4.2 million tones per annum during 1994-1995, while it was 3 million tones per annum during 1993-1994. The total area under garlic cultivation may be about 70 thousand hectares. The major garlic producing areas are Gujarat, Maharashtra, Uttar Pradesh, Karnataka, Tamil Nadu, Andhra Pradesh and Madhya Pradesh.

During 1995-1996, India exported 0.0364 million tones of garlic valued at 46.3 million rupees.

Garlic is reported to be rich in vitamins (Nanda, 1972). Garlic contains all the essential amino acids. Cystine, histidine and lysine are present in higher proportions in garlic proteins than in the proteins of common cereals. Two sulphur amino acids such as cystine and methionine were detected in the roots of garlic (Sugii *et al.*, 1964).

Allicin and allein have been considered as marker components for the evaluation of garlic (Jansen *et al.*, 1987). Allicin is unstable, thermally labile and sensitive to acid and alkali. It is a colourless oil, approximately 2.5% soluble in water and is soluble in organic solvents. It is widely used as one of the most important biologically active compounds produced by garlic having antibiotic and antimutagenic properties. The action of allicin is considerably more bacteriostatic than bactericidal (Cavallito *et al.*, 1944). Garlic juice containing allicin has been used in the laboratory experiments to stop the growth of cancer in mice. Synthetic allicin also had inhibitory effect on the development of cancer cells (Yow *et al.*, 1988). Garlic is also a rich source of highly available selenium, which is thought to account in part, for garlic's antioxidant and cancer preventive effects. Some growers add selenium to the soil to enhance garlic's selenium content. (Yang *et al.*, 1997).

The common onion (*Allium cepa*) is an excellent plant for the assay of chromosome aberrations after chemical treatment. Other species of *Allium* like *A.carnatum*, *A. fistulosum* and *A. sativum* have also been used (Grant, 1982).

The test materials used for the present investigations are *Allium cepa* and *Allium sativum*, which will be used to evaluate the cellular damage both at chromosomal and molecular level induced by five commonly used organophosphorus pesticides. We are being increasingly exposed to harmful

chemicals and radiations present in our environment. Due to the wide spread use of an increasing number of chemicals in agriculture and the resulting contamination of food with an increasing variety of pesticide residues imposes several problems. A number of adverse reactions have occasionally been observed after routine treatment with various organophosphorus compounds in domestic animals and plants. Evaluation of the cytogenetic and biochemical effects of these chemicals is of great importance since their extensive use could be detrimental to the ecosystem in general and plants in particular. Chemically induced chromosomal anomalies have been used as a rapid indicator of potential mutagenic, carcinogenic and teratogenic activity of environmental mutagens. There are evidence for a correlation between chromosomal damage and toxic effects of pesticide treatment. Thus plant and animal ecosystem are adversely affected by the application of pesticides. Apart from the dangers of acute and chronic poisoning, the possible mutagenic effects of such contaminations could threaten the genetic health of coming generations, and is therefore of high importance. Cell division, nucleic acid metabolism and protein synthesis are the primary field of action of pesticides, because these processes are essential for continued plant growth and development. When these agrochemicals interact with genetic materials, causing DNA alteration in the germ cells, they may harm the future generations.

The present work was undertaken to analyze the cytotoxic and biochemical effects of the five commonly used organophosphorus pesticides such as Ethion, Malathion, Metacid, Nuvan and Rogor on the root tip meristem of *Allium cepa* and *Allium sativum*. The test materials *Allium cepa* and *Allium sativum* were chosen since these are favourable materials for making good chromosome preparations and for assessing cytological damages quite clearly.

REVIEW OF LITERATURE

Kochuthressia M. V. "Cytotoxic and biochemical effects of some organophosphorus pesticides in Allium cepa L. and Allium sativum L." Thesis. Department of Botany, University of Calicut, 2005

REVIEW OF LITERATURE

A. CYTOTOXIC EFFECTS

Pesticides are a group of chemicals, which are used to combat the attack of pests. The use of pesticide was a new era in the application of man-made chemicals in the control of pests, which increased the production of food and helped in the eradication of diseases, but this great achievement has resulted in injury and death of a variety of forms of life. The toxic compounds get distributed by a variety of means and build up concentrations in the soil and water and finally reach human beings. In this pathway they leave high degrees of injuries to the life processes. Levan (1938) reported the classical test for the effect of chemicals on plant chromosomes. He used the root tips from bulbs of *Allium cepa* L. in an assay system.

According to Frey–Wyssling (1938), the chromosome anatomy theory seems partially responsible for chromosome movement or the chromosomes possess energy in the form of adenosine triphosphate (ATP) necessary for their movement.

Sax (1940) opinioned that if two break occurs in the same chromosome, the process of rejoining may form a ring. Anaphase bridges may be formed due to unequal exchanges or dicentric chromosomes. The occurrence of breaks at the same locus and their lateral fusion leads to the formation of dicentric chromosomes. The dicentric chromosome is pulled equally to both the poles at anaphase and bridge is formed.

Darlington (1942) found that the cytotoxic studies, gained much importance towards the middle of the 20th century. He studied the chromosome morphology, gene action, nucleic acid chemistry and metabolism and thus found out the cause of chromosome abnormalities.

Barber and Callan (1942) studied the colchicine-induced prophase–metaphases which may be attributed to the inhibition of the spindle formation.

Colchicine is known to cause delay in the division of centromere. The chromosomes would thus remain nearly in their arrangement as they were during the prophase stage. Amato (1948) observed the C-metaphase type.

Kostoff (1948 a, b) have found the multinucleate cells in BHC treatment. Ennis (1948) carried out the influence of the well-known herbicide 0-isopropyl-N-phenyl carbamate 'IPC' on meristematic cells of different plants.

The delay in the appearance of aberrations at mitosis has been reported for a large number of chemical compounds tested on *Vicia* roots. These chemicals include Nitrogen mustard by Ford (1949), β-propiolactone by Swanson and Merz (1959), Ethyl alcohol, Ethyl methane sulphonate and Myleran by Reiger and Michaelis (1960 a, b) and N-nitroso-N-methyl-urethane by Kihlman (1960).

Amato (1949) studied the cytological effects of many of the insecticides and found their relative effects on the chromosomal structure and behaviour in *Allium cepa* and *Vicia faba*. From their work, it was also evident that effects on chromosomes might lead to genetic changes in the affected plants or their progeny.

Doxey (1949) has reported the occurrence of multipolar spindles in onion and *Secale cereale* after root treatment with IPC (0-isopropyl-N-phenyl-carbamate). He attributed this action to partial suppression of spindle action. Tri/tetra polar cells and increased number of chromosomes were observed in some of the root tip cells. These may probably be due to change in physiological milieu by the organophosphorus pesticides. Kihlman and Levan (1949) reported the suppression of cell plate formation following exposure to chemicals like caffeine.

Amato (1950) studied the effects of different kinds of pesticides, insecticides, fungicides and other chemicals used in agriculture for the protection of crop against damages caused by pests, insects, fungi etc. These agrochemicals

are known to induce mitotic inhibition and chromosome damage in *Allium cepa*, *Vicia faba*, *Gossypium*, *Hordeum*, and *Drosophila*. Morgan (1950) has suggested that chromosome aberrations may also lead to asynapsis and non-homologous pairing.

Smith and Srb (1951) reported the appearance of aberrations at mitosis for a large number of chemical compounds tested on *Vicia faba*. These include β-propiolactone. Smith (1951) found that multivalent of different configurations occurs in various irradiated plants including *Hordeum*. Hindmarch (1951) has noted the delay in the appearance of interphase nuclei in mitosis and changes in the duration of the mitotic cycle in plants. Darlington and Mc Leish (1951) reported that stickiness might be due to degradation or depolymerization of chromosomal DNA.

According to Yakar (1952) a number of organochlorine pesticides have been reported to induce C-mitosis. Fragmentation was observed on cytological examination of the deformed seedlings of *Vicia faba* grown from seeds dusted with a commercial fungicide containing chloranil. Derscheid *et al.* (1952) found that the progeny does not readily inherit certain morphological malformations.

Bullought (1952) opinioned that energy required for mitosis is produced during a particular stage of the mitotic cycle. It has not been established that when metaphase begins, but it onsets when prophase ends. Regular application of herbicides to seed producing crop plants has been cited as a possible source of genetic change leading to instability (Unraw and Larter, 1952).

Mc Leish (1953) found the mitotic inhibition produced by MH (Maleic hydrazide) in root tips of *Vicia faba*. Revell (1953) showed that G_1 phase was sensitive to the chemicals.

The autoradiographic studies of Howard and Pelc (1953) showed that the interphase period in *Vicia* root-tip cells could be divided into three phases. The

pre DNA synthesis phase (G_1), DNA synthesis phase (S) and post-synthetic G_2 phase. Steffensen (1953, 1955) observed that spontaneous chromosome breakage is greatly increased when calcium or magnesium is deficient in the culture medium in which the plants are grown.

Bruhin and Warner (1954) tested the widely used insecticide Folidol. The insecticides such as Gammaxane, Lindane, DDT and various phenolic derivatives have also been known to produce cytological abnormalities in plants. They also used the insecticide 5-5-dimethyl dihydro-resorcein dimethyl carbamate on *Vicia faba*.

Hoffman-Berling (1954) opinioned that the chromosomes move under the influence of ATP, since ATP, sugar and protein metabolic pathways are interrelated. Alteration in sugar and protein synthesis may also affect chromosome movement.

Wolff and Luippold (1955) have suggested that GA promotes protein synthesis and causes reduction in frequency of chromosomal aberrations. Bhattacharya (1955) studied Systox, which caused chromosome fragments in root tip cells of *Vicia faba* with 0.001% to 0.1% concentrations.

Weinbach (1956) has found that most of the phenolic herbicides inhibit oxidative phosphorylation. Calora (1956) observed the application of insecticides viz. Parathion, Tetra ethyl pyrophosphate (TEPP) and Toxaphene on low land rice followed by the reduction in the percentage of panicle injured by *Leptocorisa acuta* and increase in yield. There was no insecticide damage to the plant at the time of application but the leaves of the treated plants yellowed more rapidly than untreated ones, when the rice was in the late dough stage.

Eigsti and Dustin (1957) evaluated that colchicine produces C-mitosis. This effect is known to be due to the action of colchicine in preventing anaphase movement by inhibiting spindle formation. Ockey (1957) examined that mitotic

index has been known to be affected after treatment with various chemicals. Hadder and Wilson (1958) conducted a cytological assay to find out the action of C-mitotic and prophase poison. They classified the antimitotics as prophase poisons and C-mitotic agents.

For a number of chemicals, it has been shown that induced chromosome breakage is specific and localized. Myleran and 8-ethoxy caffeine observed by Moutschen Dahmen and Moutschen Dahmen (1958) have been shown to induce breaks preferentially at various specific locations on the chromosomes of *Vicia faba.*

Sharma and Chaudhuri (1959) have studied Gammexane (Hexa chlorocyclo-hexane) effect on chromosomes. This induced inactivation of spindle mechanism, condensation of chromosomes, chromatin bridges, multipolar spindles and reduction in mitotic index.

Amer (1960) proved the merostathmokinetic effect of formulated Sevin and compared with that affected by Gammexane and chloral hydrate. The star-metaphase type was also observed. This metaphase type has been considered as being a fore–step of the complete disturbance of the spindle. Sax (1960) shown that the chemical mutagen induced abnormalities is known in a number of plants. Mc Manus (1960) studied *Allium cepa* with kinetin, IAA, maleic hydrazide and GA. Suneson and Jones (1960) found that the induced mutant characters were easily transmitted to the progeny.

Sparrow (1961) has reported the fragmentation of chromosomes after treatment with ionizing radiations and chemicals. Farwqui (1961) suggested that the fate of lagging univalents is that they may either be included in the developing nuclei or may be excluded to form one or more micronuclei. According to Kihlman (1961) the formation of binucleate or multinucleate cells

in treated materials may be due to disorders leading to the inhibition of cytokinesis and revealed that several chemicals are known to have this property.

The well-known chemical mutagen ethyl methane sulfonate (EMS) was used for comparative purposes. Ehrenberg *et al.* (1961) found that a seed treatment of 0.33% EMS for 24 hours caused approximately 50% X-sterility in *Hordeum vulgare*. Amoore (1961a, b) reported that the cell division in pea roots depends upon the presence of oxygen and can be arrested by the lack of oxygen.

Kihlman (1962) examined that the cell division containing chromosomal aberrations began to appear in lateral root tips of *Vicia faba* about 3 hours after immersion of the roots in solution containing FUDR (Fluorodeoxyuridine) in concentrations of 0.5 μM or higher. The aberrations consisted mainly of gaps and chromatid breaks. Aberrations involving rejoining of isochromatid breaks with sister union exchanges were extremely rare. Concentrations of FUDR that produced chromosome breakage also had a strong inhibitory effect on mitosis.

Taylor *et al.* (1962) reported the chromosomal aberrations of 5–fluorodeoxiuridine FUDR in *Vicia faba*. The most remarkable of the result was that they did not find any evidence of rejoining of broken ends after treatments with FUDR. They also suggested that the chromosomal lesions are due to interruption in DNA replication in cells, which are just finishing the phase of synthesis when affected by the analogue.

Morrison (1962) tested several carbamates including isopropyl N – phenyl carbamate, which are known to be mitotic poisons. Ethyl carbamate "Urethane", cyclohexyl carbamate and 2, 3 – dichlorallyl di-isopropyl thiol carbamate "Avadex", leads to inhibition of mitosis. Gimenz-Martin and Lopez – Sacz (1961) examined that the oxygen defficiency cause delay in the onset of division by arresting cells at metaphase. Auerbach (1962) tried to investigate that most of the fragments lead to the formation of micronuclei. These are true mutagenic

effects, which may lead to the loss of the genetic material. Barthelmess and El-Kabarity (1962) tested methyl benzoate, which possess both stathmokinetic and merokinetic activities.

Evans and Savage (1963) investigated the X-ray results where, most of the irradiated G-cells yield chromosome aberrations and chromatid type changes are mainly induced in S and G_2. These chemically induced aberrations has led to the suggestion that although the cell may be sensitive to the initiation of aberrations only in early interphase, the actual production of the aberrations occurs some stage in the development.

Veleminsky and Gichner (1963) investigated the increased use of pesticide for insect, weed and disease control in the past and has focussed attention on the fact that certain agricultural chemicals may cause damages in the genetic constitution of organisms, similar to those produced by radiations. Moutschen Dahman and Moutschen Dahman (1963) reported that EMS produces few chromosomal aberrations in barley.

Lawley and Brooks (1963) reported that alkylating agents are known to cause chromosomal breakage by binding to DNA regions rich in GC pairs causing these to become unstable. The chromotoxic effects of the organophosphorus pesticides may be attributed to their phosphorylating and alkylating properties.

Olah (1963) has shown that purified Digitonin acts as a mitostatic agent that selectively dissociates formation of the mitotic spindle from the phragmoplast. A selectively suppressed spindle characterizes Digitonin mitosis and leads to the development of a garbled phragmoplast, which produces a geometrically upset, branched cell wall system associated with the restitution tetraploid nucleus.

Hussein and Hussein (1963) have reported that the C-mitotic configurations induced by the pesticide like action of colchicine may be explained by disturbances in the synthesis of proteins, nucleic acids and antagonism between these substances. The analysis revealed that this chemical is quite effective in producing aberrant cells even at very low doses.

Epel (1963) found complete inhibition of mitosis when ATP level dropped below the 50% of normal level. Hence it may be presumed that the chemicals, which affect ATP and sugar synthesis by creating anoxia condition or by other names, exert much effect on chromosomal movement.

Hakeen and Amer (1964) examined "Rogor", "IPC", and "Duphar" and found that they differ in their mode of action on meiosis. They induce multipolar anaphase and telophase in addition to the other types of anomalies. Natarajan and Upadhya (1964) reported EMS to produce breaks preferentially at known heterochromatic regions in the chromosomes of *Vicia faba*.

Amer (1965) studied the effect of the insecticide N-methyl-1-naphthyl carbamate on mitosis and observed that the roots of *Allium cepa* which were treated for different periods of time with solutions of pure and formulated 'Sevin' prepared at 22^0C possessed mero-kinetic tendencies while those prepared at 60^0 C showed stathmokinetic tendencies. Continuous treatment with the insecticide for 24 hours nearly arrested mitosis.

Abraham (1965) has shown that the fertilisers like ammonium sulphate, ammonium phosphate, ammonium nitrate and potassium chloride produced chromosome breakage and other abnormalities in cell division in *Allium cepa*. Jain and Bask (1965) found that the chromosome aberrations might be due to certain cryptic structural changes.

Bartels and Wolf (1965) studied the effects of "Amitrale" which interferes with purine synthesis and they affect nucleic acids. Such action could explain the induction of chromosome aberrations by such chemicals.

The effects of aflatoxins on cell division and chromosomes have been the subjects of numerous investigations since severe cytotoxic effects were reported by Lilley (1965). According to Cottam (1965) the plant protection chemicals are potentially hazardous in the human environment; their use has been markedly increased during the last few years. While the use of these chemicals to control pests is indispensable, the frequent and indiscriminate use of these chemicals may have many undesirable secondary consequences.

Mann and Storey (1966) have proved that the inhibition of cell division and the presence of deformed nuclei in root tip cells, were causes for the death of roots, which culminates into seedling mortality.

Cummins *et al.* (1966) reported that the proteins, which determine the duration of transition from metaphase onwards, are concerned with the transformation of chemical energy into the mechanical work of mitosis. This assumption is also strengthened by the fact that agents, which suppress the energy generating processes like glycolysis, respiration and oxidative phosphorylation, inhibit cell division.

Hammouda *et al.* (1966) examined the effect of the insecticide N-methyl-l-naphthyl carbamate on the vegetative and reproductive growth of the plant. Experiments of Liao and Hamlton (1966) have shown that labelled 2, 4-D are localized in the chromosomes in root tips of *Vicia faba* and *Allium cepa*. It is known that 2, 4-D combined with proteins forms chemical complexes (Butts and Fang, 1956). Thus the induction of chromosome aberrations by 2, 4-D is possibly a result of its action on nucleic acids directly or indirectly.

Kihlman (1966) has shown that the inhibition of oxidative phosphorylation results in inhibition of cell divisions. This is because the development of the mitotic processes requires energy. Most of the energy required by the cell is provided by oxidative phosphorylation in mitochondria in the form of ATP.

Lawley (1966) investigated the correlation between alkylating, mutagenic, carcinogenic and other deleterious biological activities of chemicals. A rapid standard assay, the NBP colour test has been developed for the assessment of alkylating agents.

Pickett-Heaps (1967) observed that the mechanism of cytokinesis in plant cells involve the formation of new cell wall with the help of different organelles such as microtubules, Golgi-complex and possibly mitochondria. Accumulation of these organelles in the vicinity of phragmoplast was rarely seen in the treated cells. Sometimes incomplete cell wall formation in patches was also seen. These alterations suggest that isoproturon interferes in the process of cytokinesis by affecting the function of microtubules and Golgi complex and leads to the formation of binucleate cells. The application of pesticides may also affect certain biochemical processes in a cell, which eventually upset the chromosome division cycle (Liang *et al.*, 1967).

Reddy and Rao (1968) studied the cytological effect of two organophosphorus systemic insecticides viz. Dimecron –100 and Rogor– 40 with different concentrations on the broad bean plant, *Vicia faba*. Chromosome and chromatid breaks, deletions, fragments and anaphase bridges were noticed in both metaphase and anaphase.

Amer and Ali (1968) have shown that spraying with certain pesticidal chemicals produce meiotic aberrations in *Vicia faba*. Scott (1968) investigated that the mitotic inhibition caused by herbicides has been attributed to blocking of

mitotic cycle during interphase that may result from a prolonged G_2 period or to the inhibition of DNA synthesis.

Prasad and Pramer (1968) studied the induced chromosome aberrations in plants and in yeast by the compounds such as bis-dithane (dizine bis dimethyl dithio carbamate), ethylene bis-dithio carbamate and Ferban (iron dimethyl dithio carbamate). It is also known that some inorganic arsenic compounds act as mutagens in bacteria.

Lofroth *et al.* (1969) proved that Dichlorvos and Methyl parathion have the alkylating properties and mitodepression seem to be related to the phosphorylating and alkylating properties of a chemical. Hepler and Jackson (1969) studied certain antimitotic chemicals such as colchicine, vimblastine, deltametrin and few carbamates and found that they act upon microtubules and induce mitotic aberrations.

Herich (1969) discovered that zinc, which is essential for normal growth of plants, in excessive amounts inhibit cells from passing from interphase to prophase and induced disturbances in spiralization of chromosomes. Amer and Ali (1969) noted the effect of ortho and para-nitro-phenol in *Vicia faba* and found that mitotic index was low at 0.025% to 0.1% concentrations. They also observed cytomixis in the mitosis of *Vicia faba*. Morse and Yanofsky (1969) proved that caffeine increase the chromosome aberrations induced in *Vicia* and Ascites tumour cells. Most of the pesticides have been reported to alter the chromosome number acting as spindle poison, causing chromosome stickiness or serving as cross wall inhibitors (Liang *et al.*, 1969).

Ravindran (1970) proved the cytological effects of commercial Folidol in *Allium*. It has been found that Folidol produces many chromosome abnormalities such as anaphase inhibition, C-mitosis, chromatid and chromosome breakage, abnormal chromosome separation and micronuclei formation.

Epstein *et al.* (1970) reported the possible mutagenic nature of the organophosphate such as trimethyl phosphate. Subsequently the potential genetic effects of selected organophosphates were investigated in a number of test systems (Dyer and Hanna, 1970).

Leffler *et al.* (1971) found that the herbicide 2, 4-D and Amitrole induced chromosome aberrations. It has been shown that 2, 4-D at low concentrations (<100 p.p.m.) stimulates RNA synthesis but not DNA synthesis, and at concentrations greater than 100 p.p.m, enhances both DNA and RNA synthesis as judged by DNA and RNA polymerase activity.

Amer *et al.* (1971) found that the chromosomes spread irregularly all over the cell in the case of disturbed metaphase, anaphase and telophases. Such type may be due to the disturbances of the spindle apparatus. Disturbed type dominated also in both *Vicia faba* and *Gossypium barbadense* after seed soak and root-treatments with Sevin. Epstein and Lagator (1971) proved the mutagenicity of some common pesticides and have recommended barley as one of the test materials for screening the mutagenicity of a chemical because of various advantages including induction of distinct chlorophyll deficient mutants.

Fishbein (1972) evaluated the use of organophosphorus insecticides in current agricultural practices. The efficacy of these insecticides in the better exploitation of plant species of economic importance is well known. The potentialities of these pesticides as mutagenic and /or carcinogenic agents to the non-target organisms are well known.

Voogd *et al.* (1972) studied eight organophosphorus compounds used as insecticides because of their powerful inhibition of insect cholinesterase activity. Parathion, Methyl parathion, Malathion, Dimethoate diazinon, Dichlorvos, Methyl oxydemeton and Bidrin were the insecticides used. The alkylating

properties of these compounds are similar to those of monofunctional alkylating agents.

Ashwood-Smith *et al.* (1972) observed weak mutagenesis of *E. coli* by DDVP and concluded that the agar plate method was inappropriate for detecting a weak mutagen that was both volatile and rather toxic. Dyer and Hanna (1972) reported the mutagenic activity of TMP (trimethyl phosphate) and briefly compared the TMP and DDVP in a similar test. Initial mutagenic screening tests were carried out using the '8 pot methods' advocated by Clarke (1971).

Ahmed and Grant (1972) studied the cytological effects of the insecticide Phosdrin (mevinphos) and the herbicide Bladex on root tips of *Tradescantia* and *Vicia faba* and compared with those of the chemical mutagen ethyl methane sulfonate (EMS). The frequency of chromosomal aberrations induced by both pesticides deviated significantly from that of the control.

Mohandas and Grant (1972) investigated the cytological effects of 2, 4-D and Amitrole in 12 species. (*Tradescantia* clone 02, *Allium cepa, Vicia faba, Triticum aestivum, Triticum dicoccum, Hordeum vulgare, Secale cereale, Centaurea jacea, Cirsium vulgare, Chrysanthemum lancanthemum, Plantago major and Erigeron canadensis*). Ethyl methane sulfonate (EMS) was used as a positive control. The cytological abnormalities induced in root-tip cells by 2, 4-D and Amitrole included chromosome bridges, fragments, lagging chromosomes and chromatin bodies. 2, 4-D also induced chromosome contraction and C-mitosis.

Bridges *et al.* (1972) had previously obtained rather variable results using the agar plate spot test, which has been very successful in characterizing the mutagenicity of the fungicide Captan. According to Dean (1972), Malathion did not induce mutation in bacteria. No increase in chromosome breakage was found

in Chinese hamster cells *in vitro* exposed to Malathion at concentrations of 50-400 μg/ml.

Ames *et al.* (1973) revealed a high correlation between mutagenicity and carcinogenicity. Adequate mutagenicity testing on pesticide might be useful for the evaluation of their toxicities. Because of the massive amount of information about the mutagenicity of pesticides, tight regulations are now being implemented for the registration of pesticides.

Swietlinska *et al.* (1973) tested the root tips of *Vicia faba* incubated in cycloheximide at concentrations of 0.3 – 50 μg/ml. It inhibits the incorporation of leucine by 40-100% within 2 hours. A depression in the incorporation of thymidine was observed after 2-hour incubation in cycloheximide at 1 μg/ml. In root tips exposed for 2 hour to cycloheximide at 1 μg/ml, the mitotic activity of cells was severely depressed within 15 hours of recovery. Metaphases appearing after 20 hours carried infrequent aberrations of the chromatid type. Cycloheximide at this concentration had no effect on the yield of aberrations induced by the alkylating agents diepoxybutane (DEB) and N-ethyl-N-nitrosourea (ENU) when applied as post-treatment.

Amer and Farah (1974) observed the cytological effects of the insecticide "Rogor" on the mitosis of *Vicia faba* and *Gossypium barbadense*. Both pure and formulated "Rogor" inhibited cell division in *Vicia faba*, as it is evident from the effect of seed –soak and root – treatments.

Van Bao *et al.* (1974) reported the chromosome aberrations in Malathion intoxicated humans. The Malathion intoxicated patients had incorporated the highest doses of insecticide in the entire patient group. The relatively low toxicity of Malathion may be connected with its metabolism. Malathion in mammals is converted by hydrolysis of carboethoxy group mainly to "Malathion acid" which is only a weak cholinesterase inhibitor. Malathion acid is still a

triester of phosphoric acid and as such a potential alkylating agent. Kada *et al.* (1974) revealed the mutagenicity of Captafol and Captan.

Beck and Obey (1975) revealed the induction of sister chromatid exchanges (SCEs) by chemicals. It has been found to be a sensitive way in which potential mutagenic effects can be assessed. Latt (1975) also observed that the sister chromatid exchanges are increased at concentrations, that produce no chromosome breakage. There appears to be a correlation between induced SCEs and mutations at one gene locus in mammalian cells *in vitro* by Carrano *et al.* (1978).

There is an increasing interest in rapid screening system for the detection of potential mutagens or carcinogens in the environment. Bacterial tests with or without metabolic systems have shown that most carcinogens are mutagenic by Ames *et al.* (1975). Cytogenetic studies have also shown that mutagens and/or carcinogens induce chromosomal aberrations in plant cells by Kihlman (1975) or in mammalian cells *in vivo* by Goetz *et al.* (1975).

Carter (1975) studied adriamycin, one of the best antibiotics of the anthracycline class, very recently introduced in cancer chemotherapy. Even though its pharmacological and biochemical aspects are known at present, some adverse side effects, which the drug causes in the recipients, cause serious health problem.

Amer and Farah (1976) studied the effects of the insecticides "Rogor" and "Duphar" on the mitosis of *Vicia faba* and *Gossypium barbadense*. Spraying *Vicia faba* plants with saturated IPC solution at the flowering stage induced a relatively high percentage of abnormal PMCs (pollen mother cells). The transmission of chromosomal aberrations to the following generation was found to be very low.

Prasad and Das (1977) observed in *Vicia faba* that low concentration of GA enhances cell division whereas high concentration inhibits. Mitchison (1977) investigated that a successful completion of mitosis is a result of integrated harmonious functions of the cell as whole. Presence of pesticide molecules or their derivatives may disturb the synthesis or the structure of DNA or may cause inhibition of cell entry into mitosis.

Grant (1978) pointed out that plant chromosomes are sensitive indicators to environmental pollutants and rightly suggested that the higher plant system appears to be an excellent indicator of the cyto-toxic, cytogenetic and mutagenic effects of environmental mutagens. Therefore, the plant system must be accepted as a first tier assay system for detection of the possible genetic damage resulting from the use of environmental chemicals.

Marshall and Roberts (1978) have shown that single acute exposures for a duration of 4 to 24 hours in chloropyrifos at concentrations ranging from 0.02 to 20 μg/ml did not have statistically significant chromosome damage though the rate of increase of sister chromatid exchanges did not reach significant levels at 0.02μg/ml compared to one of the two controls. It seems that chloropyrifos is an insecticide that causes relatively limited chromosome damage at low dosage exposures.

According to Deserres (1978), the herbicides are capable of inducing genetic effects on plants. Many studies have demonstrated the mutagenic action of herbicidal solution on seeds, seedlings and growing plants. Plant assay system has been found to be useful for the detection of environmental mutagens. It is ironical that although herbicides are used in modern technology of crop production, they are a source of potentially hazardous substances in the human environment. Oku (1978) tested the seedlings of *Allium cepa*, with Maleic

hydrazide at pH 4.8, 5.8 and 6.8. Chromosomal aberrations at anaphase and telophase were observed in root tip cells in all experiments.

Matsuoka *et al.* (1979) investigated a metabolic activation system with rat liver microsome fraction, plus cofactor S_9 mix was applied to chromosomal aberration tests *in vitro* for the screening of chemical mutagens or carcinogens in the environment. Dialkyl nitrosamine induced chromosomal aberrations in Chinese hamster cells (CHL) when treated with S_9 mix. The increase of chromosomal aberrations in CHL varied with experimental conditions. Ann *et al.* (1979) tested the effect of non-toxic concentrations of Malathion on sister chromatid exchange frequencies.

Amer and Farah (1979) studied the cytological effect of Leptophos. It is an insecticide of moderate mammalian toxicity, the effect of which was tested on root mitosis, seed germination, root and shoot lengths of *Vicia faba*. Both treatments affected a significant percentage of abnormal mitosis. Seed-soak treatment for 48 and 72 hours showed marked inhibition of cell division.

Sakamoto and Takahashi (1979) found that the three fungicides, Mancozeb, Benomyl and Carboxin had an inhibitory effect on cell division in *Allium cepa*. Zart *et al.* (1979) treated the half bulbs of *Allium cepa* with three different concentrations of fungicide and an insecticide for 24 hour after 6 days pre-soaking in water. Difference in the percentage of normal cells between treated root tips and control were observed at the two higher concentrations of the insecticide and the middle concentration of the fungicide. The highest dose of fungicide inhibited cell division.

Bansal and Sen (1979) examined the germinating bulbs of *Allium cepa* with roots 20mm long after placing in 0.01% solutions of ethyl methane sulphonate (EMS) and 2, 4-D. Chromosome bridges were induced in root tissue by EMS but no such bridges were found when 2, 4-D was applied before EMS;

the frequency of chromosome bridges was reduced when 2, 4-D was applied after EMS. However, with 2, 4-D treatment, a high percentage of polyploid and polytenic nuclei occurred, where as if 2, 4-D were followed by EMS the frequency of such nuclei was much reduced.

Mishra and Sinha (1979) determined the influence of Malathion on mitotically dividing onion root-tip cells. The mitotic index decreased in the presence of the organophosphate. The percentage of cells in prophase increased at lower concentrations, where as the percentage of cells in metaphase, anaphase and telophase increased at higher levels. The abnormalities observed included stickiness, fragmentation, laggards, ana-telophase bridges and multipolar spindles. Kabarity and Nahas (1979) reported that the frequency of polyploidy was reduced when the Trifluralin treatment was withdrawn. Trifluralin also induced c-tumours in onion root tips.

According to Datta and Chedda (1980) pesticide mediated alkylation not only of guanine but also of thymine, cytosine and uracil and subsequent base replacement is perhaps the most common mechanism of mutagenesis in micro-organisms, cells in culture, or in experiments carried out in *in vitro* conditions. However, almost unmodified pesticide molecules are available for causing the damage. In higher organisms, the pesticide induces a series of enzyme-mediated biotransformations and these bio-transformed forms happen to be mutagenic.

Nasar and Singh (1980) observed the possible cytogenetic effects of Carbaryl on target and non-target organisms. The treatment of onion root meristems induced an accumulation of interphase nuclei with a larger diameter, and a subsequent decrease in the index of cells with smaller nuclei. It is concluded that Carbaryl depresses mitosis by arresting cells at G_2 without affecting DNA synthesis.

Beuret (1980) found that Pendine-thalin exerts a marked depressive effect on mitosis in the root meristem of *Allium cepa* and disturb chromosome division. The depressive effect starts at a concentration between 10 and 1μM and the functional disturbance can be observed at 0.1 μM. The effects of treatment also show inhibition of root growth and thickening of the meristematic area.

Jagoda (1980) studied the effect of selected herbicides (Limuron, Simazine, Alipur and Liro) upon root tip meristems of *Allium cepa*. The mitotic activity of cells treated with herbicides at 1g/litre markedly decreased in comparison with the control. Herbicides induced structural and numerical aberrations of chromosomes and inhibited the function of the karyokinetic spindle, as well as cytokinesis.

Kozera (1980) discovered the influence of the fungicide Benlate and Topsin-M on the mitotic process in root meristems of onion. Benlate and Topsin-M, applied at five different concentrations for 24 and 48 hours, reduced the mitotic index of root tip cells, in comparison with that in untreated controls.

Pandita *et al.* (1981) examined the cytological effects of Bavistin on root tips of *Allium cepa*. High concentrations of Bavistin given for one day or more increased the frequencies of both cell division and chromosome aberrations. Chen *et al.* (1981) discovered the induction of sister-chromatid exchanges (SCEs) and cell cycle delay in Chinese hamster cell line V_{79} after treatment with eight organophosphorus pesticides. In addition, these effects were also studied using one of the eight organophosphorus pesticides in 2 human lymphoid cell lines.

Mandel and Basu (1981) investigated the treatments with IAA and GA_3 singly and especially after X-ray irradiation and produced significant reduction in mitotic index in root meristem of *Allium cepa*. X-rays induced chromosomal aberrations. The percentage of protection ranged from 18-55 with IAA and 14-

53 with GA_3. These growth hormones were considered to have reduced aberrant cells by providing protection against initial radiation damage and also by stimulating restitution presumably by affecting DNA synthesis and also by stimulating protein synthesis respectively.

Wooder and Wright (1981) showed the alkylating properties of Dichlorvos and Methyl parathion. The chromotoxic effects of these organophosphorus pesticides may be attributed to their phosphorylating and alkylating properties.

Sahu *et al.* (1981) noted the effects of fungicide Dexon and its derivatives on root meristems of *Allium cepa*, *A. sativum* and *Vicia faba*. Mitodepression was observed at 1000 p.p.m. of Dexon and 750 p.p.m of DMPDA (dimethyl propane diamine) in *Allium cepa* and at 500 p.p.m of Dexon and 250 p.p.m of DMPDA in *A. sativum*. Both chemicals induced C-metaphase and inhibited cytokinesis in *A. cepa* and *A. sativum*.

Reddy and Rao (1981) revealed the effects of insecticides "BHC" and "Nuvacron" on chromosomal mechanism in relation to yield and yield components in chilli (*Capsicum annum* L.). Both BHC and Nuvacron produced abnormalities in dividing pollen mother cells and the frequency of these abnormalities was higher in Nuvacron than in BHC.

According to Hutson (1982), the fats and lipids present in the body/cells combine with pesticide metabolites to produce a conjugate type of chemical and it is believed that these conjugates have the real mutagenic nature.

Reddy and Rao (1982) studied the considerable effects of the herbicides namely Lasso and Basagran at higher dose levels on chilli crop. It was observed that both germination and survival were effected in all the treatments of Lasso and Basagran. The injurious effect of Lasso on germination and survival was more than in Basagran. Similarly both Lasso and Basagran treatments produced

meiotic aberrations and the frequency was markedly higher in Lasso treatments than in Basagran.

Badr and Elkington (1982) found the antimitotic and chromotoxic activities of isoproturon in *Allium cepa* and *Hordeum vulgare*. Isoproturon considerably reduced mitotic activity and exerted a disruptive action on the spindle apparatus resulting in C-metaphase figures, doubled chromosome number, chromosome lagging and multipolar anaphases and telophases. Isoproturon also induced chromosomal breakage, stickiness and bridges. The abnormalities produced in onion and barley was similar, but comparison of the frequencies of abnormalities in both plants indicated that onion was susceptible to the mitodepressive and chromotoxic actions of the herbicides.

Oku (1982) observed the effects of pre-treatment with AET (Amino ethyl-iso-thio urea) on the chromosomal aberrations induced by MH in root tips of *Allium cepa* L. The frequency of chromosomal aberrations in anaphase and telophase root tip cells was examined. AET pre-treatment occasionally reduced the frequencies of MH-induced telophase bridges and chromosomal fragments but did not alter the lagging of chromosomes.

Grant (1982) found that the common onion (*Allium cepa*) is an excellent plant for the assay of chromosome aberrations after chemical treatment. Other species of *Allium* such as *A. carinatium, A. fistulosum* and *A. sativum* have also been used. From a literature survey, 148 chemicals were tabulated that have been assayed in 164 *Allium* tests for their clastogenic effects. A review of the various types of chromosomal anomalies and the pesticides that produced them was also given. Moreover, a link between chromosomal anomalies produced by these compounds gave mutation has been confirmed by several workers (Gichner *et al.*, 1982). The use of chromosomal aberrations induced by pesticides in crop plants, is therefore, being accepted as indicators of genetic damage. Malathion

and one of its break down products Malathion acid are alkylating agents to nucleic acid while others insist that the potential for damage is significant and advocate further testing.

Amer and Farah (1983) observed the cytological effects of the insecticide Dursban or Chloropyrifos on the mitosis of *Vicia faba*. Seed-soak and root-treatments with different Dursban concentration induced a significant percentage of abnormal mitosis. The percentage of which increased as the concentration of the experimental agent increased. They also revealed the cytological effects of the insecticide Dipterex or Trichlorphon on root mitosis, meiosis and pollen viability of *Vicia faba* plant. Root treatment for four hours caused distinct decrease in the mitotic index indicating mitotic inhibition. The types of abnormalities observed in meiosis were the same as those observed in mitosis except chromosome stickiness, which was observed in a high percentage in meiosis only.

Badr (1983) evaluated the mitodepressive and chromotoxic activities of two herbicides Carbetamex and Paradone plus in *Allium cepa*. Chromosomal aberrations produced consisted of C-metaphase and C-anaphase formations, chromosome stickiness, bridges, lagging, and multipolar anaphases and telophases. Both herbicides induced the formation of multinucleate cells, micronuclei and chromatin bridges in interphase cells. Chromatin lost its staining ability or appeared as dense granules, and nuclei became vacuolated. It is concluded that these herbicides have mutagenic potential.

Somasekhar and Arekal (1983) studied the chromosomal aberrations induced by electroplating wastewater. When bulbs of *Allium cepa* were grown in the presence of electroplating waste water (EWW) containing Cu, Cr, Ni, and other heavy metals at lower concentrations and cyanides at concentrations

ranging from 1 to 80%, mitotic index decreased and frequency of chromosome aberrations increased with EWW concentration.

Pandita and Khoshoo (1984) revealed the clastogenic and turbogenic effect of the systemic insecticide Thimet and found that below 0.1% it will not cause any genetic damage. Higher doses are both clastogenic and turbogenic.

Somasekhar (1984) worked on the fungicide Benlate in the root meristem of *Allium cepa* and proved the mitodepressive and clastogenic effects. Benlate inhibited cytokinesis and spindle formation and reduced the mitotic index also. Somasekhar *et al.* (1984) studied the cytological effects of fungicide Topsin in *Allium cepa*. Topsin induced C-metaphase and spindle abnormalities and inhibited cytokinesis in onion.

Somasekhar and Gouda (1984) studied the effect of a fungicide Vitavax on *Allium*. It induced spindle abnormalities, inhibited cell plate formation and exhibited antimitotic activity in onion. Clastogenic effects were pronounced at a concentration of 500 mg/litre.

Mansour (1984) revealed the cytogenetic effect of Tribunil on plants and chromosome aberrations have been recorded in root tips of *Vicia faba*. The role of ascorbic acid (Vitamin-C) as antimutagen, anticlastogen and anticarcinogen have been evaluated by Shamberger (1984) under *in vitro* conditions, and has been found to decrease the incidence of carcinogen induced gene mutations.

Kaur and Grover (1985) proved the cytological effects of eight organophosphorus pesticides such as Anthio, Diazinon, Dursban, Ekalux, and Methyl parathion, Phendol, Rogor and Sumithion. The pesticide treated samples revealed several cyto and chromotoxic effects. The dose-response curves were found to be positively correlated in all the pesticides.

Amer and Farah (1985) studied the effect of the insecticide Methamidophos on root-mitosis of *Vicia faba*. The insecticide showed no

significant effect on the mitotic index meanwhile it induced a highly significant percentage of cells with chromosome aberrations.

Reddi and Reddi (1985) observed the degree of cytological aberrations either in mitosis or in meiosis as one of the dependable criteria for estimating the effect of mutagen. Esteran-48 exerted a marked mitodepressive action on mitosis and induced a number of chromosomal aberrations.

Nandi (1985) tested the cytogenetic effect of some mercuric fungicides such as Ceresin, Agrosan and mercuric chlorides on onion root tips. Different chromosomal aberrations were also noted. El-Shehaby and Mohamed (1985) discovered the side effect of fungicide on onion plants. Treating onion plants with Benlate, affected the amounts of chemical constituents during the 6 weeks after the last spray. Sugars and phenolic compounds fluctuated, excepting conjugated phenols, which decreased with fungicide treatments. Most amino acids and total free amino acids decreased, while histidine increased with all treatments.

Murthy and Hammanthu (1985) proved the clastogenic and mitoclastic effects of benzimidazole derivatives. The colchicine like effects obtained suggest that, benzimidazole may be used as a pre-treating agent for chromosome analysis.

Rao and Rao (1986) discovered chromosomal aberrations induced by pesticides Endosulfan and Rogor in mitotic cells of *Allium cepa* L. The Mitotic index was also decreased when these pesticides were applied at 1000, 3000 and 5000 p.p.m. to dividing cells. Nainala (1986) tested the effect of Metaxuron on the meiotic behaviour in *Allium cepa*. It inhibited meiosis and the percentage of anomalies increased as the dose increased.

Airapetyan (1986) observed the mutagenic effect of the fungicides Ridomil, Benlate and Ploridrel on seedlings. The spontaneous chromosome

aberration frequency was significantly increased by treatment with higher concentration of each fungicide. The main types of aberrations were single fragments and chromatic bridges.

Rao *et al.* (1986) have shown clastogenic and turbogenic effects of organophosphorus pesticides on *Allium cepa* root meristem. Methyl parathion was the most effective clastogenic as well as turbogenic agent, inducing a wide range of chromosome abnormalities. Malathion had strong clastogenic properties, but Quinalphos was a weak clastogenic and turbogenic agent.

Devadas *et al.* (1986) studied the four organophosphorus insecticides TARA-909, DDVP, Phosphomidon (PMN) and Monocrotophos (MCP) on germination, survival and meiotic behaviour in *Capsicum annum*. Chromosomal aberrations and pollen sterility were dose dependent and there was an apparent relationship between frequency of chromosomal damage and pollen sterility.

Amer and Mikhael (1986) studied the effect of the insecticide Rotenone on root-mitosis of *Vicia faba*. Earlier, Meisner and Sorensen (1966) as well as Barham and Brinkley (1976) have studied the effect of Rotenone on mammalian cells on Chinese hamster cultured cells. Sadhu and Waters (1980) in their review on some chemical pesticides reported that Rotenone is a potent spindle tubule poison and suggested an evaluation of its mutagenicity.

Amer and Ali (1986) studied the effect of the pure insecticide Dichlorvos on the root-mitosis of *Vicia faba* plant. Dichlorvos treatments induced cells with chromosome abnormalities, which increased in number as the concentration of the insecticide was increased. Chaurasia and Sinha (1986), tried to investigate urea and single super phosphate induced cytological changes in *Allium cepa*.

Somasekhar *et al.* (1987) discovered the cytological effects of a fungicide Rizolex in *Allium cepa* L. This systemic fungicide caused partial impairment of

spindle formation in young onion roots and is consequently defined as a mitotic poison with radiomimetic action.

Badr and Ibrahim (1987) tried to investigate the effect of herbicide Glean on mitosis, chromosomes and nucleic acids in *Allium cepa* and *Vicia faba* root meristems. Treatment with Glean inhibited mitosis and depressed DNA and RNA contents in both species. Long exposure to the higher concentrations proved lethal. A wide range of mitotic chromosome abnormalities was observed, their frequency generally increased with dose and duration of treatment.

Vaughan and Vaughan (1987) showed that propyzamide disrupts mitosis. Bright-field light, Nomarski differential interference, immunofluorescence and transmission electron microscope showed that many of the effects of propyzamide on onion roots were similar to those produced by colchicine and trifluralin. The disruption of the mitotic spindle by propyzamide was unique.

Airapetyan (1987) tried to investigate the cytogenetic activity of some insecticides on the chromosome apparatus of onion. When root tips were treated with Anthio and Dursban at 0.01 and 0.05% concentrations, a significant increase in the frequency of chromosome aberrations was detected.

Rao (1987) tried to investigate the effect of two agrochemicals Dicofol and Paraquat on mitotic divisions in *Allium cepa* root meristems. Growing root tips of onions were exposed to Dicofol and Paraquat and produced a number of chromosomal aberrations.

Prakash and Lakshmi (1988) evaluated the cytological effects of two fungicides Bavistin and Deltan in Chilli (*Capsicum annum*). Both seed germination and seedling survival were effected with an increase in concentration. The mean chiasma frequency per cell was decreased with increased concentration of treatments.

Badr (1988) investigated the effect of Dithane and Denmart at concentrations of 4 p. p. m. to 500 p. p. m. on root meristems of *Allium cepa*. High concentrations of Dithane gave rise to lower prophase frequencies while those of Denmart reduced the frequencies of anaphase and telophase, relative to other mitotic phases. Both compounds induced colchicine–type metaphase configurations.

Ekatin, fenitrothion, phorate and the known mutagen MNNG (N-methyl-N-nitro-N-nitrosoguanidine) were studied using an *in vivo* chromosomal aberration bioassay in root meristems of *Allium* and *Hordeum* by Grover (1988). The pesticides induced both physiological type aberrations (C-mitosis, despiralization, lagging chromosomes, multipolar cells) and clastogenic effects (chromosomic breaks, ring chromosomes, chromatin bridges and micro nuclei).

Rao *et al.* (1988) tried to investigate the cytological effect of herbicide Asulam and insecticides Chlorpyrifos and Endosulfan on *Allium cepa* root meristems. The pesticides induced a wide range of chromosomal aberrations including chromosome and chromatin breaks, chromatin bridges, fragments, micronuclei and sticky chromosomes.

Sofradzija *et al.* (1988) observed mutagenic and genotoxic effects of some herbicides (Tordon and Arbogal) in meristem tissue of *Allium cepa*. Both herbicides under all conditions reduced the mitotic coefficient significantly. The genotoxic and mutagenic effects of both herbicides increased with concentration and exposure time.

Somasekhar (1988) proved the mitodepressive and clastogenic effects of the fungicide Brassical in root meristem of *Allium cepa*. The fungicide inhibited cytokinesis, resulted in the accumulation of binucleate and ultimately polyploid cells. Clastogenic effects such as breaks, gaps, exchanges, bridges, fragments and laggards were observed. In many cells, laggards led to the development of

micronuclei. Sattar and Vahidy (1988) revealed the genotoxic effects of pesticides Endrin and Benlate on root-mitosis of *Allium cepa*.

El – Khodary *et al.* (1989b) tested the effects of the herbicide Goltix on root mitosis of *Allium cepa*. Exposure of root tips of onions with Goltix induced statistically significant chromosome aberrations mainly C-metaphase and bridges.

Grover *et al.* (1989) investigated the mutagenic effects of Carbaryl, a contact insecticide with slight systemic properties. Carbaryl did not enhance significantly the frequency of histidine revertants in any of the strains of *Salmonella* tested. However, Carbaryl induced both clastogenic and physiological type of chromosomal aberrations. The spectrum of chromosomal aberrations included C-mitosis, stickiness, vagrant chromosomes, polyploidy, multi-polarity, delayed anaphases, end to end joining of chromosomes, chromosome breaks, ring chromosomes and anaphase bridges. Transferring the Carbaryl treated bulbs to distilled water for 24 and 48 hours reduced the frequency of chromosomal aberrants.

Papes *et al.* (1989) studied the *Allium* test response to Cyanazine. The root tip cells were examined for mitotic activity, spindle organization and chromosome aberrations. Percentage of mitosis was lower than in the control after 3 hour, at all concentrations.

Hidalgo *et al.* (1989) have shown that Propham and Chlorpropham induced abnormal mitosis and growth inhibition in *Allium cepa* roots. Abnormalities were found in meristematic cells of *Allium cepa* roots exposed to the herbicide Propham at a range of concentrations (10^{-1} to 10^{-5} M) and durations of treatment (1 to 6 hours). Chlorpropham was also tested under similar conditions. The results, which show root growth inhibition, mitotic index decrease, multipolar anaphases, anomalous chromosome separation and anaphase

emigration, might result from an alteration of the microtubule organizing centres and spindle organisation induced by these herbicides.

Kumar and Sinha (1989) found the threshold dose of cytogenetic toxicity of Lindane, Malathion and Metacid in *Allium cepa* root-tip cells. Sinha *et al.* (1989) examined the cytological effects of Phosalone on root meristem of *Allium cepa* L. The mitotic index gradually decreased with increasing pesticide dose and treatment time. The percentage of chromosomal abnormalities decreased with increasing recovery time, especially in the cells treated for 24 hours with a recovery period of 48 hours.

Genotoxic effects of 2, 4 - Dichlorophenoxy acetic acid (2, 4-D) using multiple genetic assay systems of plants were studied by Kumari and Vaidyanath (1989). Chromosome aberration frequency increased with increased dose in onion root tips treated with 2, 4-D at 25-100 p. p. m. A study of M_1 and M_2 revealed an increase in mutation frequency with increasing mutagen dose.

Younis *et al.* (1989) revealed the physiological and cytogenetic effects of Phosphine gas in *Allium cepa* L. Phosphine appeared to increase the rate of mitotic inhibition and chromosome aberrations, although it had no effect on seed protein content.

Abraham and Devi (1989) reported that magnesium sulphate, which is used as a fertilizer and in the preparation of different nutrient media, utilized for *in vitro* cultures, inhibits normal spindle formation and cytokinesis. As a result, binucleate cells, polyploid cells and multinucleate cells were formed.

Kumar *et al.* (1989) studied Phosalone on root meristems of *Allium cepa*. The pesticide induced mitostatic effects on root meristem cells and the mitotic index gradually decreased with the increase in concentration and time of treatment. Mutagenic effects were noticed with abnormalities like chromosome

breakage and erosion, sticky bridges, laggards, multipolarity, polyploidy, clumping and pycnosis.

John *et al.* (1989) examined the genotoxicity of the organophosphorus insecticide Malathion based on human lymphocytes in cultures. The potential of the compound as a genotoxic agent was determined on the basis of chromosome damage and rates of sister chromatid exchanges in human lymphocyte culture. A trend of higher rates of chromosomal aberrations was observed revealing statistical significance generally at the higher concentrations. The sister chromatid exchange rates were highly and significantly elevated at higher concentration. Malathion therefore should be viewed as a potentially genotoxic agent, particularly for those who come in contact with the compound repeatedly.

Nelson *et al.* (1990) investigated the genotoxicity of the organophosphorus insecticide Chloropyrifos based on human lymphocytes. Organophosphorus compounds such as Chloropyrifos are popular insecticides because of their effectiveness as cholinesterase inhibitors, high degree of water solubility, and rapid biodegradation. It is therefore imperative to continue to assess potential genetic damage from limited exposure to organophosphorus insecticides.

El-Khodary *et al.* (1990) investigated the cytological effects of the herbicide Tribunil on *Allium cepa* root tip cells. The result obtained showed that Tribunil inhibits spindle formation and is mitodepressive in function. The herbicide also induced clastogenic effects such as laggards, bridges, breaks and fragments and therefore it is recorded to be a potential mitotic poison.

Chauhan and Sundararaman (1990) studied the possible effects of isoproturon on the root growth, macromolecular biosynthesis and mitosis in the root meristem cells of *Allium cepa*. The compound exerted dose-dependent root growth retardation. Inhibition of macromolecular biosynthesis as observed with

the treatment of isoproturon possibly lead to the mitodepression. Induction of various cytological abnormalities suggests that isoproturon is highly turbogenic (spindle poison) rather than clastogenic. Moreover, induction of chromosome breaks in addition to various mitotic abnormalities may lead to mutagenic events.

Sobhi and Haliem (1990) proved the effects of herbicide Rancho on root mitosis of *Allium cepa*. A drastic drop in mitotic activity was observed in all of the treated roots. A variety of chromosomal abnormalities were induced by the herbicide. The proportion of abnormalities increased with the increased herbicide concentration and the period of treatment.

Eissa and Eissa (1990) discovered the cytological effects of the insect growth regulator Fenoxycarb on mitotic cells of onion. The potential effect of the insect growth regulator as a mitotic poison was clearly demonstrated by spindle disfunction leading to C-metaphase, disrupted anaphase, polyploidy and unequal chromosomal distribution.

Haliem (1990) tested the cytological effects of the herbicide Sencor on mitosis of *Allium cepa*. All concentrations tested decreased cell division of onion root cells. Sencor resulted in a change in the frequencies of the different mitotic stages. As the concentration of the herbicide increased, the frequencies of prophase, anaphase and telophase decreased with a corresponding increase in the frequency of metaphase.

Klein (1990) studied the C-mitotic action of the insecticide Ambush 25 EC in *Allium cepa* L. The effect on mitosis was estimated immediately after the insecticide action and after 24 and 48 hours. Ambush 25 EC disturbed functioning of the spindle, causing C-mitotic changes in the meristem cells of onion roots. This effect was greater at lower temperatures and was also affected by the duration of treatment. Grover *et al.* (1990) found the genotoxicity of pesticides on plant systems. The results of testing for the potential of 18

pesticides including 14 systemic and nonsystemic insecticides, 3 systemic fungicides and 2, 4-D showed that all these induced chromosome aberrations in onion, barley and rat bone-marrow cells.

Lehmen *et al.* (1990) tried to investigate the effects of Terbutol on onion root tips. Star anaphases, were the chromosomes are drawn with their centromeres to both poles and resulted in a star bust of chromosomes, where the predominant form of mitotic abnormality noted in root tip squashes of the Terbutol treated roots.

Bellami *et al.* (1991) recorded a linear correlation in *Allium* between the concentration of surfactants and percentage of abnormalities. Several workers had carried out similar types of genotoxic effects of different chemicals on different plant materials.

Hada *et al.* (1991) found that administration of vitamin C, either concurrently or as pre and post-treatment to the pesticide exposure, was very much helpful in minimising mitoinhibition and clastogeny, induced by two organophosphorus pesticides Malathion and Rogor. The concurrent treatment with vitamin C was more effective than the two other modes of its supplementation.

Sharma and Goutam (1991) found that chemicals are toxic to the genetic material of chromosomes, by inducing duration related mitotic anomalies. Patil and Shirashyad (1991) proved the effect of Methyl parathion and Phosphanidon on mitotic chromosomal aberrations in some vegetable seeds such as okra, onion, bean and guar. Okra exhibited the most cytotoxicity while guar exhibited the least.

Samad *et al.* (1992) tried to investigate the cytological effects of Endosulfan (Thiodan) on root mitosis of *Allium cepa* and *Vicia faba* and found a chromosomal abnormality of 9.68 and 15.55% in Endosulfan at a concentration

of 0.3% after one and two hours of treatment respectively. In the case of *Vicia faba,* the chromosomal abnormality was 14.0 and 19.72 % after one and two hours of treatment respectively.

Ahmad and Yasmin (1992) studied the effect of Trimiltox and Methyl parathion on the mitosis of *Allium cepa.* There was a gradual increase in the percentage of chromosomal aberration, with the increase of chemical concentrations. Chromosomal aberrations such as micronuclei, fragments, laggards and single and multiple bridge formation were observed, with Methyl parathion inducing more aberrations than Trimiltox. Methyl parathion produced comparatively more chromosomal aberrations than Trimiltox.

Soh and Yang (1993) studied the effect of plant growth regulators on mitotic chromosomes in *Allium cepa* L. Rank *et al.* (1993) evaluated the genotoxic potential of Roundup and glyphosphate isopropylamine. The most frequent aberrations observed could be characterized as disturbances of the spindle.

Hada and Sinha (1993) studied the genotoxic effect of Rogor at various concentrations used in agriculture, in terms of mitotic index in onion root-tip cells, chromosome abnormalities and meiotic index in mice and lethal mutation rate in *Drosophila.* The pesticide could cause mitotic as well as meiotic inhibition, increase the clastogenicity rate and induce lethal mutations. The effect of L-ascorbic acid was studied after giving the vitamin concurrently with the pesticide. The cytogenetic toxicity of Rogor was appreciably decreased by ascorbic acid.

Kara *et al.* (1994) revealed cytogenetic effects of the insecticide Cypermethrin on the root meristems of *Allium cepa.* Actively growing root tips of onion were used to evaluate the cytotoxic effects of Ronstar by Butt and Vahidy (1994). It showed a significant reduction in the mitotic index.

Abnormalities of lesser frequencies included C-mitosis and polyploidy. The study of the mutagenic effects of zinc phosphide and of mitomycin-C (an alkylating agent) in the presence of activated waters was done by Gavrila *et al.* (1994). Sister chromatid exchange indicated that chromosomal aberrations arose with a rate which paralleled the time in which the mutagen acted, both in control and in experimental variants, using activated waters.

Butani and Shukla (1994) investigated the cytological effects of pesticides on onion (*Allium cepa)* root tip. A reduction in mitotic index and reduction in germination was observed with all pesticides except Carbendazim. There was a concentration and time-dependent mitodepression. Different types of chromosomal aberrations were observed. Induction of chromosomal breaks suggested abnormalities with increase in concentration and it also indicated the genotoxic levels of respective pesticides.

Sudhakaran *et al.* (1994) found the effect of Metacid-50 on mitosis of *Allium cepa.* Clumping of chromosomes, stickiness, C-metaphases, non-orientation of chromosomes, anaphase bridges, diagonal anaphase, C-anaphase, binucleate cells and micronuclei were observed in different stages of mitosis in treated cells. Amita *et al.* (1994) discovered that the treatment with Monocrotophos and Endosulfan resulted in chromosomal and mitotic abnormalities in onion root meristem. Guha and Das (1994) studied the mitotic effects of organophosphorus pesticides on *Allium cepa* with special attention to chromosomal abnormalities.

Nagpal and Grover (1994) evaluated the cytotoxic effects of five systemic pesticides Carbaryl, Formothion, Derosal, Metasystox and Rogor by chromosomal aberration assay in root tip cells of onion (*Allium cepa*). Increase in the aberration frequency at recommended dose of all test pesticides was

statistically insignificant over the control value. A positive correlation was found between different concentrations of pesticides and frequencies of aberrations.

Ignacimuthu and Kumar (1994) observed the cytogenetic effect of Endosulfan-35 EC on *Allium cepa* by immersing root meristems in a series of concentrations. Various chromosomal abnormalities such as stickiness, clumping, breakage, bridges, laggards, multipolarity and ring chromosomes were produced. Mitostatic effects were also observed at higher concentrations. The results indicated that indiscriminate use of Endosulfan would cause irrepairable chromosome damage in onions.

Ignacimuthu and Kochuthressia (1994) studied the cytogenetic and biochemical effects of organophosphorus systemic insecticide, Monocrotophos in *Allium cepa* and *A. sativum*. The analysis of root tip cells of treated plants revealed chromosomal aberrations. There was a direct relationship between the frequency of aberrations and insecticide treatment. Besides various biochemical parameters were analysed and found that Monocrotophos interferes with many metabolic activities. The results indicate that Monocrotophos has cytotoxic properties and even act as a mutagen.6

Pandey *et al.* (1994) examined the treatment of different concentrations of Dithane M-45, Aldrex-30 and Metacid-50 and showed the positive chromotoxic effects in *Allium cepa* root meristem. Effects included high lethality for cell division, clumping, bridges, fragments, cytomixis, disturbed polarity etc. Such genotoxic effects warrant frequent use of these chemicals, which were found to have lethal effects on cell division and induced cytological disturbances during root tip mitosis. All the concentrations were capable of inducing different types of chromosomal abnormalities and the frequency of abnormalities increased in most cases, with increase in concentrations.

Datta *et al.* (1995) studied the mitostatic effects in *Pisum sativum* and *Trigonella foenum-graceum* following seed treatments with Cythion, Cytozyme, Endosulfan and Monocil. They were tested for their potency in inducing cytotoxic effects on the root tip mitosis. Efficacy of the compounds were predicted in the following order: Monocil> Cythion> Cytozyme > Endosulfan.

Kumar *et al.* (1995) found the cytogenetic toxicity and no-effect limit dose (NELD) of three commonly used pesticides in a number of test systems using a sufficient number of lower doses to characterize the dose-effect relationship. It was concluded that the NELDs were not only pesticide specific but also organism specific, tissue specific and even damage specific. Further more, the NELD values determined were so small that the real human exposure to pesticides cannot be reduced below these levels without compromising the effectiveness of pesticides in use.

Priya *et al.* (1996) evaluated Malathion treated cells and showed a reduction in the mitotic index during all doses and durations when compared to the control sets. During all treatments, the divisional frequencies were found to be reduced even at one-hour exposure, indicating that the pesticide has an immediate effect on the dividing cells. This would reveal the mitotoxicity of the test chemical.

Abraham (1997) studied the cytological abnormalities produced by muriate of potash on *Allium cepa* root meristem. Different concentrations of the fertilizer showed that concentrations ranging from 200 p. p. m. to 2000 p. p. m. produced significant increase in chromosomal aberrations and other abnormalities over the control. This study indicates that the fertilizer is capable of upsetting normal cell division if excessive amounts were present in the soil.

Ghareeb and George (1997) studied the effect of Temik on both mitosis and meiosis. The insecticide decreased the mitotic activity of *Vicia faba* root tip

cells and produced different kinds of abnormalities. These kinds of abnormalities were nearly identical in both mitosis and meiosis. Stickiness was the most common type of abnormality that resulted after all treatments. Disturbances in the mitotic phase, which have a mitotic poison effect, were also present with considerable percentage. Such phenomena indicate the toxicity of Temik and its low mutagenic potential.

Cytogenetic effects of Glyphosate and 2:1 Metolachlor-Atrazine on root tips of *Allium cepa* was tested by Kooffreh (1999). The following chromosomal aberrations were observed after the particular treatment: bridges, laggards, acentric fragments and disturbance of the spindle fibres. These aberrations were not dose-dependent. Glyphosate and Metolachlor-Atrazine were also observed to reduce mitotic activity. Mitotic inhibition values increased with increasing herbicide concentrations.

Khynriam and Prasad (1999) examined the root tips of onion treated with Cisplatin for 48 hours. A decrease in the mitotic index and an increase in the number of interphase cells were seen in Cisplatin treated root tips. An increase in the frequency of abnormal mitosis and chromosomal aberrations was also observed in Cisplatin treated groups which indicates its genotoxic effect on plant cells. The endogenous Glutathion (GHS) level in the root tips decreased significantly after Cisplatin treatment which may favour its increased interaction with cellular DNA thereby developing enhanced chromosomal aberrations and effecting cell divisions and root growth. It is suggested that the decrease in endogenous GHS may be related to the development of Cisplatin – mediated genotoxic effects in plants.

Chauhan *et al.* (1999) evaluated the cytogenetic effects of Cypermethrin and Fenvalerate on the root meristem cells of *Allium cepa*. Cypermethrin was found to be more toxic than Fenvalerate, as the frequency of aberrant cells was

much higher in Cypermethrin–treated cells. These observations indicate that spindle poisoning is the primary mechanism of genotoxic action of these insecticides. However, the low percentage of chromosome breaks also indicated the clastogenic potential of these compounds.

Sushama *et al.* (1999) studied Ultragin an allopathic drug and Tribhuvan kirti a herbal drug commonly used as analgesic and antiviral ones and compared their effects on root mitosis in *Allium cepa*. The root tips were treated with different concentrations of both the drugs Ultragin and Tribhuvan kirti, which induced mitodepression, mitotic abnormalities and structural aberrations of chromosomes. The M-phase abnormalities induced by Ultragin and Tribhuvan kirti were mitotic arrest at various stages, clumping and stickiness of chromosomes, irregular distribution of chromosomes at anaphase together with sticky bridges. Structural aberrations were manifested in the form of fragments, laggards and micronuclei.

Sabale and Mane (2000) investigated cytotoxic effects of maleic hydrazide on *Allium cepa* varieties. Higher concentrations and long term exposure to the herbicide induced significant physiological and clastogenic effects such as clumping, laggards, bridges etc. in both varieties. The variety N-2-41 exhibited a higher frequency of chromosomal aberrations than N-53. The results confirmed the mutagenic effects of maleic hydrazide on plant cells.

Hanaa *et al.* (2000) investigated the image of cytometric measurements demonstrated by a dose dependent effect of Aflatoxin B_2 (AFB_2) treatments on the components of mitotic cycle in *Vicia faba* L. root meristematic cells. The most evident effect appears to be the accumulation of cells in the G_O/G_1 phase at the expense of other phases of the cycle such as S phase, G_2 phase and M phase. These results indicate that this toxin acts as an inhibitor of cell cycle progression at the G_1 transition point. The inhibition of mitotic activity induced by AFB_2

treatments is associated with a reduction in seedling growth. Cytological examination of dividing cells revealed an abundance of dose-dependent chromosome abnormalities produced by the applied treatments of AFB_2. Chromosomal abnormalities associated with stickiness of chromosomes or due to an action on the mitotic apparatus are the dominant abnormalities induced by this toxin. The capacity of this toxin to induce clastogenic aberrations may be regarded as an indication of its genotoxic potential. This is also indicated by the formation of micronuclei in interphase cells.

Gomergen (2000) studied the effect of the herbicide 2,4-D isooctylester 48% on root mitosis of *Allium cepa*. It also caused reduction in the mitotic index, indicating mitotic inhibition and increased the frequency of abnormal mitosis. The herbicides are capable of inducing genetic effects on plants. Many studies had demonstrated the mutagenic action of herbicidal solutions on seeds, seedlings and growing plants. Plant assay system had been found useful for the detection of environmental mutagens.

Grant and Elizabeth (2001) tabulated 117 chemicals that had been assayed in 179 assays for their clastogenic effects in *Pisum*. Of the 117 chemicals that had been assayed, 65 are reported at giving a positive reaction, causing chromosome aberrations, 30 positive with a dose response, five broader line positive and 17 chemicals gave a negative response. 81% of the chemicals gave all definite positive response. C-mitotic effect was detected from treatment with 17 chemicals. In addition to the above tabulation of chemicals, 39 chemicals had been reported with an antimitotic effect.

Kalia *et al.* (2001) studied five environmental industrial chemical agents belonging to three separate groups such as Aziridines, Metapa and Thiotepa, nitrosocompounds like MNG and the alkaline sulphonic esters MMS and EMS. They compared for their mutagenicity and related cytogenetical effects on two

wheat varieties. On the over all basis, highest number of abnormal cells and shattering of chromosomes were observed under Metapa, followed Thiotepa. The other two chemicals agents, MNG and MMS also exhibited larger chromosomal abnormalities than EMS and control.

Giri *et al.* (2002) reported the genotoxic effects of Malathion, which induced *in vivo* micronucleus. The genotoxic potential of technical grade Malathion (2.5, 5, 10 mg/Kg) induced significant dose dependent increase in the frequency of micronuclei in the bone marrow cells of chicks. A significant decrease in mitotic index was observed for the highest dose tested. In the peripheral blood cells, significant increase in the frequency of micronuclei could be observed for the higher doses (5, 10 mg/kg) tested. The results indicate that technical grade Malathion may be considered as a mutagenic compound in chicks and is cytotoxic at higher doses.

Nagpal *et al.* (2002) tested *in vivo* chromosomal aberration assay employing root tip cells of *Allium cepa* L. This was used to assess genotoxic effects of two azo dyes, Acid violet 3 and Direct blue 2B. A wide spectrum of cytotoxic effects induced by both dyes included spindle inhibition, stickiness, delayed anaphase, laggards and vagrant chromosomes (physiological effects), and chromosomal breaks, bridges and ring chromosomes (clastogenic effects). Delayed anaphases, chromosomal breaks and bridges were the most common cytotoxic effects induced by these dyes. With Acid violet-3, stickiness and C-mitosis were noticed at a concentration above 62.50mg/100ml. Vagrant chromosomes and ring chromosomes were noticed only in a few cells. With Direct blue 2B, stickiness was noticed with all concentrations of the dye; C-mitosis was noticed at a concentration above 31.25mg/100ml. Laggards and ring chromosomes were noticed in a few cells. Statistical analysis revealed that there

existed a positive correlation between different concentrations of dyes and frequencies of aberrations.

Chandra *et al.* (2002) evaluated clastogenic effects of Trifluralin, a dinitro aniline herbicide on root tip cells of *Vicia faba*. Mitotic metaphase indices and frequency of chromosomal aberrations observed in the *in vivo* assays revealed a wide spectrum of cytotoxic effects of test chemical. These cytotoxic effects showed a linear relationship in terms of concentrations and treatment time of Trifluralin. The spindle disruption ability of Trifluralin was quite pronounced alarming the safe handling of the chemical during agricultural operations.

Kumar and Tripathi (2003) studied the comparative cytological effects of mercuric chloride and lead nitrate on cells of *Allium cepa* with respect to the cell cycle response, mitotic index and chromosomal aberrations. The results indicated that both the chemicals had depressive effects on the mitotic index as compared to their respective controls. The percentage reduction in mitotic index values was higher in case of mercuric chloride treatment as compared to lead nitrate. The chromosomal aberrations like stickiness, laggards, chromatin bridges, C-metaphase, fragmentation and binucleate cells were increased with the increasing concentration of the tested chemical. Although, both mercuric chloride and lead nitrate share a common range of aberrations, the former induced greater percentage of clastogenic abnormalities where as the latter induced mitotoxic abnormalities in abundance. The results showed that mercury has more mutagenic potentialities than lead.

Sreedevi and Bindu (2004) observed the cytotoxic effect of aluminium sulphate on root tip cells of *Allium cepa*. The metal was found to decrease the mitotic index and induce interphase nuclear death, vacuolation and nuclear polymorphism in cells of treated bulbs. Other abnormalities encountered were, disturbed metaphase and anaphase, clumping, chromatid bridges, sticky bridges,

ring chromosomes, change in normal chromosome morphology and multiple groupings at anaphase and telophase.

B. BIOCHEMICAL EFFECTS

Zaheer (1948) investigated that the DNA and RNA exist in a cell in complex or in combination with other macromolecules such as proteins and carbohydrates. Quantitative analysis of DNA and RNA enables us to understand the nature of chromosomes of various rice varieties. Biochemical composition of rice is influenced to some extent by genetic and environmental factors.

According to Amato (1952), the drastic effect of Aziridines could be due to its ability for immediate binding and reactivity at many sites, which could be considered analogous to 'prophase poisoning'. It is thus inferred that normal function of the cells are suppressed either due to impairment of DNA synthesis or blockage at synthetic stage of interphase cells.

Flaks and Cohen (1959) have investigated the monophosphate of FUDR, 5-fluorodeoxy uridylic acid, as a strong inhibitor of the enzyme thymidylate synthetase. When the supply of thymidylic acid is removed from the cell, deoxy ribonucleic acid (DNA) replication stops.

Wolff (1959) suggested that GA promotes protein synthesis and cause reduction in frequency of chromosomal aberrations.

Sharma and Sharma (1960) conducted the investigations on spontaneous and chemically induced chromosome breaks. In their opinion, chromosome breaks were caused by disruption of nucleic acid cycle of the cell together with non-synchronized nucleic acid metabolism at different gene sites. They also suggested that the upset of the nucleic acid metabolism ultimately results in disturbed protein reduplication causing chromosome to break at different loci.

Matsuura and Tanifuji (1962) found that the inhibitors of protein synthesis such as chloramphenicol and CHM to influence the rejoining of chromosome

ends broken by irradiation. However, the effect of these two inhibitors include repair of aberrations induced by ionising radiation in *Vicia*.

Clark-Walker and Linnene (1966) reported the well-known difference of cycloheximide in the mode of action of inhibitors of protein synthesis. Chloramphenicol inhibits mitochondrial protein synthesis, but not cytoplasmic protein synthesis in yeast. In *Euglena*, this antibiotic more effectively inhibited the protein synthesis in chloroplasts than in cytoplasm.

Maaloe and Kjeldgaard (1966) discovered that inhibition of protein synthesis causes inhibition of RNA synthesis and unbalanced DNA synthesis that is known to offer greater radio resistance. Kihlman (1966) indicated that potent DNA inhibitors are primarily responsible for reduction in mitotic activity or it could be due to inhibition of DNA synthesis or change in the oxidative phosphorylation activity.

Freeze and Bantz-Freeze (1966) examined that most of the chemical mutagens are known to induce several types of DNA alterations with different efficiencies in *E. coli*.

Schultz and Funderburk (1967), reported relation between changes in cell division activities with changes in nucleic acid and protein content among them. Mann *et al.* (1967) studied that isoproturon affects diffused chromatin and inhibited RNA synthesis in the root meristem cells of *Allium cepa*. In this regard, it is important to mention that number of other pesticides have also been shown to induce chromatin condensation and inhibit RNA synthesis.

Liang *et al.* (1967) have shown that the vital processes of the crop itself may be affected by the use of pesticides. The most serious injury is at the cytological level, where the DNA molecule and consequently the gene are altered. Regular application of pesticides to crop plant has been cited as a possible source of genetic damage leading to instability.

Vant' Hoff (1968) explained that the drop in mitotic activity which is not accompanied by the inhibition of DNA synthesis may be due to an increase in the G_2 period.

Preussmann *et al.* (1969) have studied that the organophosphorus insecticides are chemical alkylating agents. These properties and its dependence on the individual chemical structure are revealed by the NBP (nitrobenzyl pyridine) test. DNA alkylation studies have been conducted with dichlorvos only.

Buiatti and Ronchi (1969) proved the effect of protein synthesis inhibitors on the production of aberrations by chemical mutagens. According to them, cycloheximide and chloramphenicol enhances the frequency of aberrations induced by 6-methyl coumarin in *Allium*. Caffeine, which effectively inhibits protein synthesis, potentiates production of aberrations by a number of chemical mutagens in both plant and animal cells.

Lofroth (1970) studied the effect of DDVP (2, 2-dichlorovinyl dimethyl phosphate), which is the active constituent of the insecticide Vapona. It has been shown to alkylate DNA.

Ellis and Mac Donald (1970) investigated that CHM (cycloheximide) specifically inhibits cytoplasmic protein synthesis in eukaryotes and indirectly depresses DNA synthesis.

In the myxomycetes *Physarum*, a 60 minutes incubation in CHM (Cycloheximide) 10μg/ml resulted in 70% inhibition of protein synthesis and stopped DNA synthesis within 1 hour of the addition of CHM (Muldoon *et al.*, 1971). Heiner (1971) found that diethyl sulphate caused a blockage of DNA synthesis, which in turn induced a substantial mitotic delay.

Stockert *et al.* (1971) observed that the marked reduction in metabolic activity brought about by low temperatures and a drop in the intracellular ATP

pool is due to anoxia or due to the disconnection of the oxidative phosphorylation, which inhibit RNA synthesis.

Gumanov and Gumanov (1972) explained that many of the organophosphates are triesters of phosphorus or phosphoric acid and thus genetically interesting in that they may also be alkylating agents. Tomkins and Grant (1972) had attributed the inhibition of cell division by chemicals to action on DNA and RNA in the cells.

Bedford and Robinson (1972) revealed that the organophosphorus compounds such as chloropyrifos are popular insecticides because of their effectiveness as cholinesterase inhibitors, high degree of water solubility and rapid biodegradation. However, many of these compounds and their break down products are known to be alkylating agents of DNA.

Evans *et al.* (1972) found that the duration of the mitotic cycle increases with increasing nuclear DNA amounts and there is substantial interspecific variation in DNA amounts within *Allium*.

Fest and Schmidt (1973) found the acute toxicity of organophosphates for mammals and probably also for insects, which is mainly due to the blocking of cholinesterase enzymes which are in part essential and normal neural transmission. The mechanism of this blocking is phosphorylation of serine hydroxyl group in the active site of the enzyme.

Legator *et al.* (1973) observed that TMP is a simple alkyl compound used in industry and is known to alkylate *E. coli.* phage T_4B to cause chromosome breaks in bone-marrow cells of rats.

Zuk and Swietlinska (1973) reported the effect of caffeine at concentrations of 1000μg/ml, which inhibits protein synthesis in bean root tips to the same extent as does CHM (cycloheximide) at 1μg/ml but no mitotic delay. Bridges *et al.* (1973) tested the mutagenic and lethal action of methyl methane

PLATE - 1

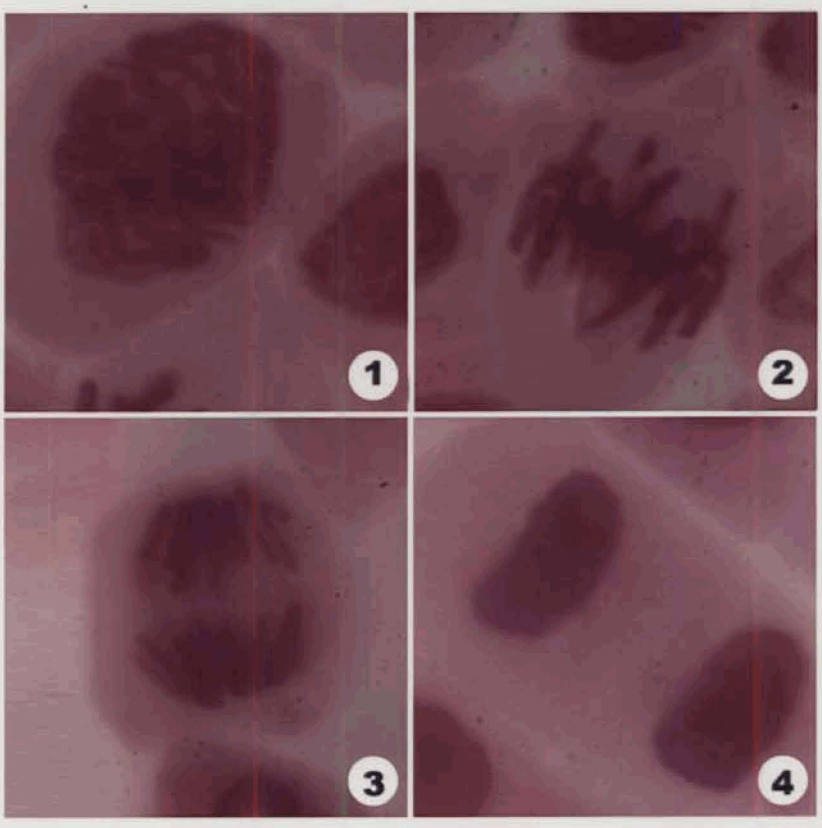


FIGURE LEGEND - Normal Stages of *Allium cepa*

1.Normal Prophase
2.Normal Metaphase
3.Normal Anaphase
4.Normal Telophase

PLATE - 2

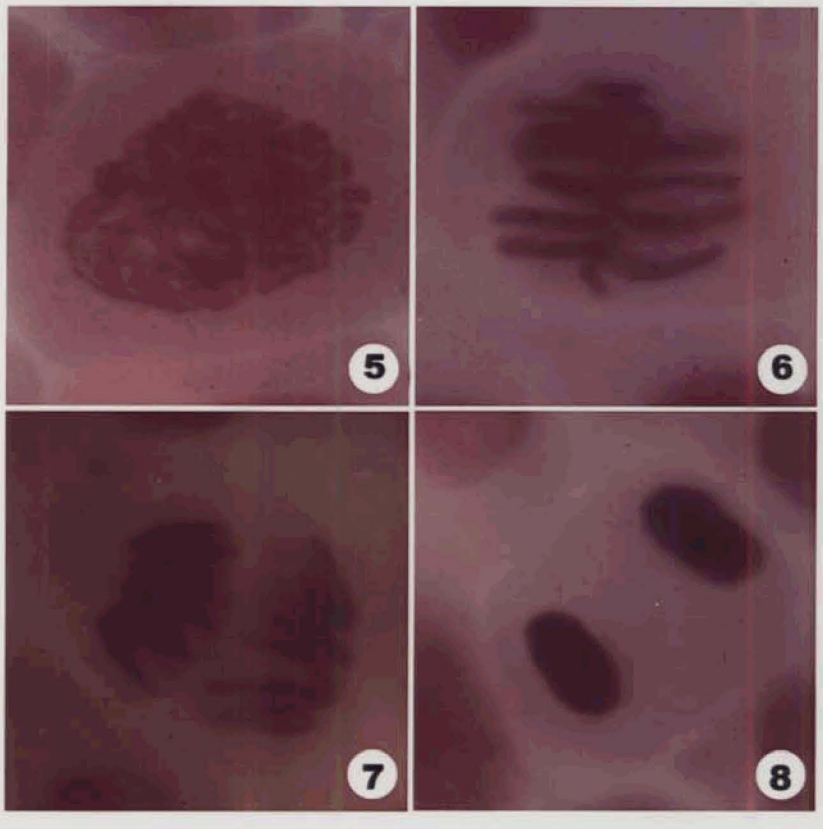


FIGURE LEGEND - Normal Stages of *Allium sativum*

5.Normal Prophase
6.Normal Metaphase
7.Normal Anaphase
8.Normal Telophase

FIGURE LEGEND

Figs. 9 - 68. Chromosomal aberrations induced by organophosphorus pesticides in *Allium cepa* (x 1000).

9. Nuclear lesions at interphase.
10. Elongated nuclei showing prominent lesions
11. Sickle shaped nucleus during interphase.
12. Interphase cell with a micronucleus.
13. Interphase cell with many nuclear fragments.
14. Unequal nuclear fragments.
15. Binucleate cell.
16. Binucleate cell.
17. Binucleate cell with two lesions in each nucleus.
18. Dumbbell shaped nucleus.
19. Multiple nuclei in a cell showing nuclear deformation.
20. Nuclear extrusion.

PLATE - 3

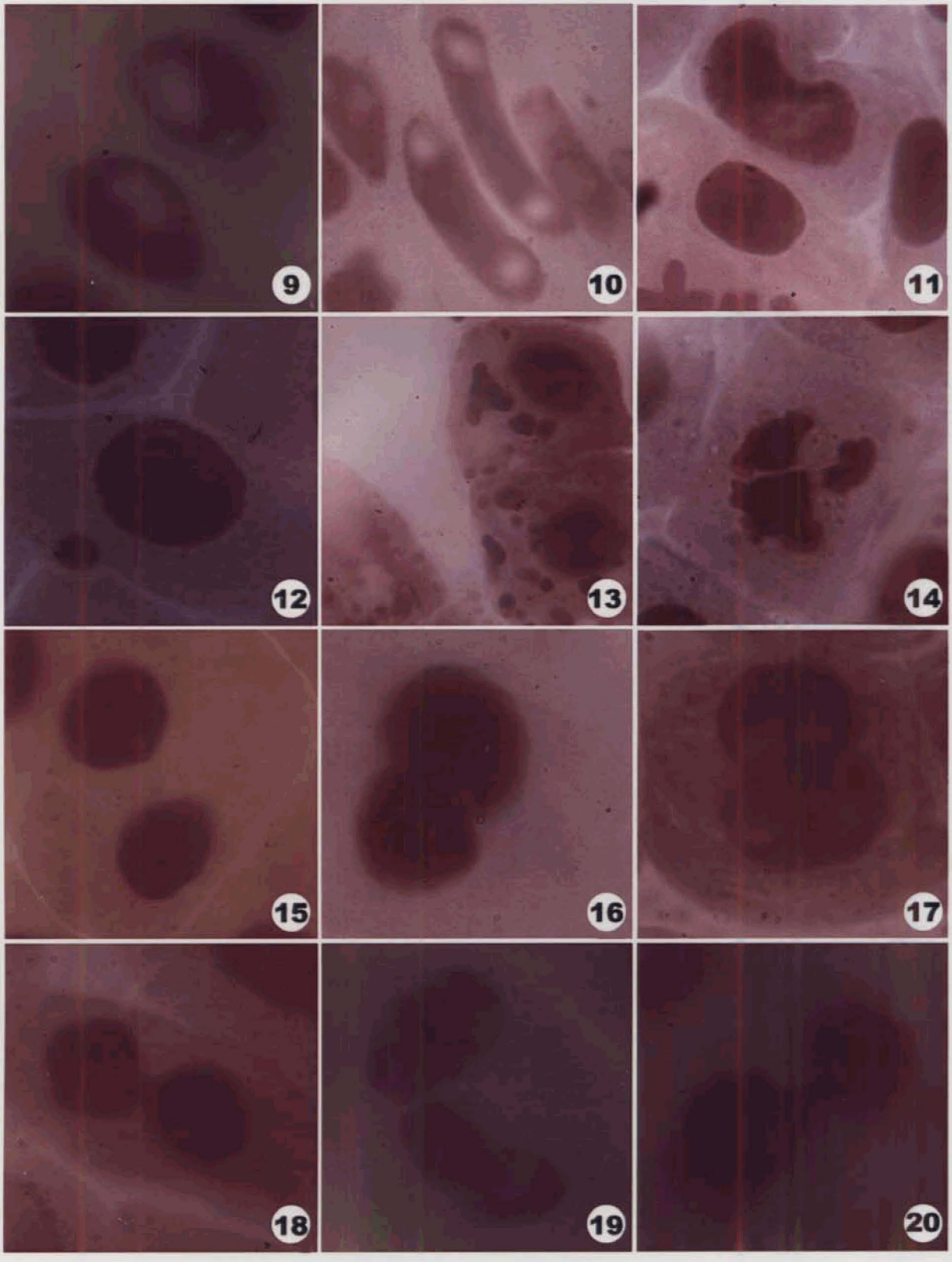


FIGURE LEGEND

21. Misorientation of chromosomes at metaphase.
22. Highly irregular chromosomes at metaphase.
23. Chromosome fragments and partial sticky metaphase.
24. Dislocation of a chromosome at metaphase.
25. Sticky metaphase.
26. Diagonal C-metaphase.
27. Diagonal metaphase showing chromosome stickiness.
28. Stickiness and dislocation of a chromosome at metaphase.
29. Star metaphase.
30. Cells at C-metaphase.
31. Clumping of chromosome at metaphase.
32. Ball metaphase with a prominent lesion.

PLATE - 4

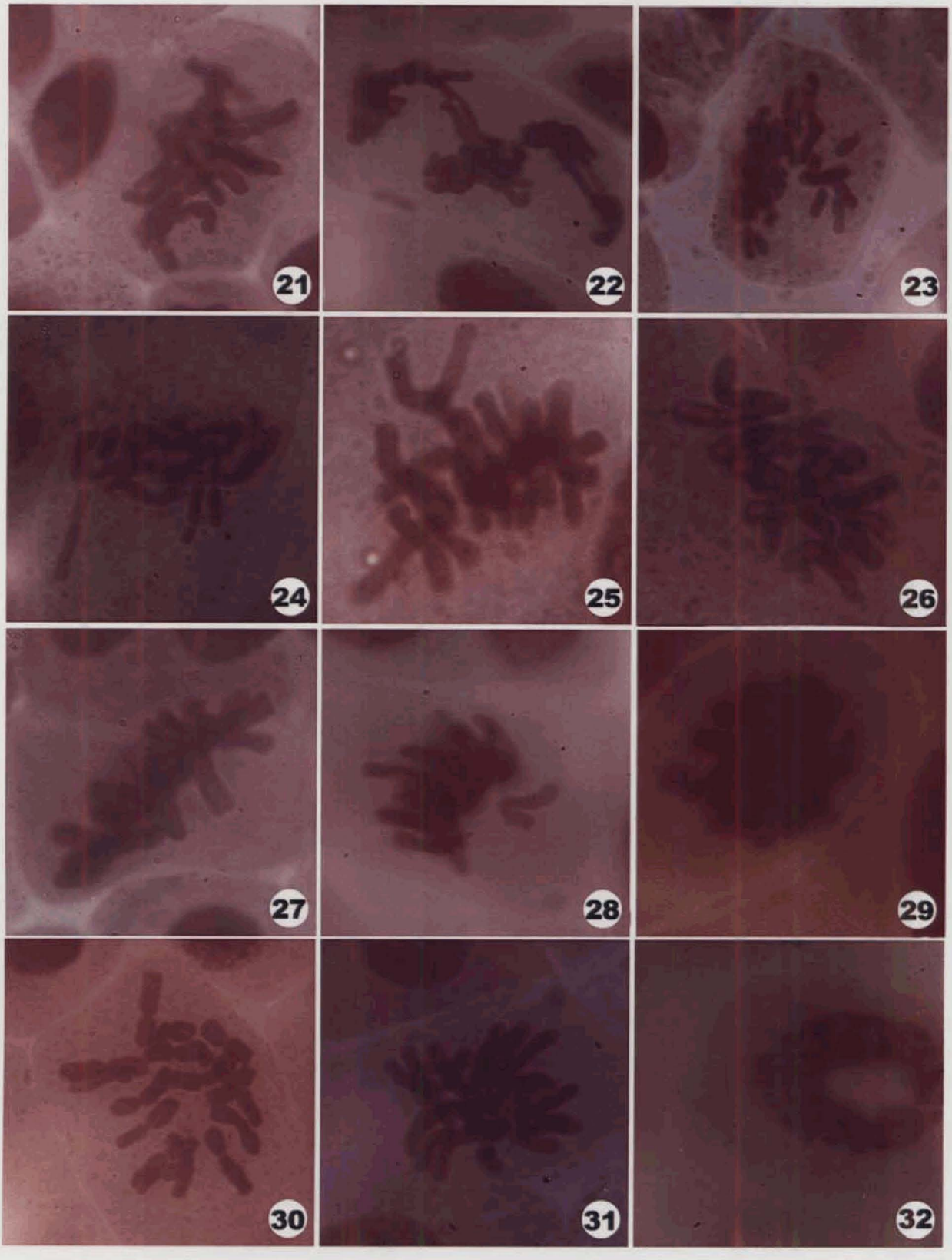


FIGURE LEGEND

33. Unequal segregation and abnormal association of chromosomes.
34. Chromosome erosion and scattering during metaphase.
35. Chromosome pulverization at metaphase.
36. Stickiness at anaphase.
37. Sticky anaphase showing multiple bridges.
38. Anaphase with a ring chromosome.
39. Multipolarity and unequal segregation of chromosomes.
40. Sticky anaphase with bridges.
41. Stathmo-anaphase.
42. Chromosome stickiness at anaphase with a bridge and a ring.
43. Stickiness and bridges at anaphase.
44. Bridges and non-orientation at anaphase.

PLATE - 5

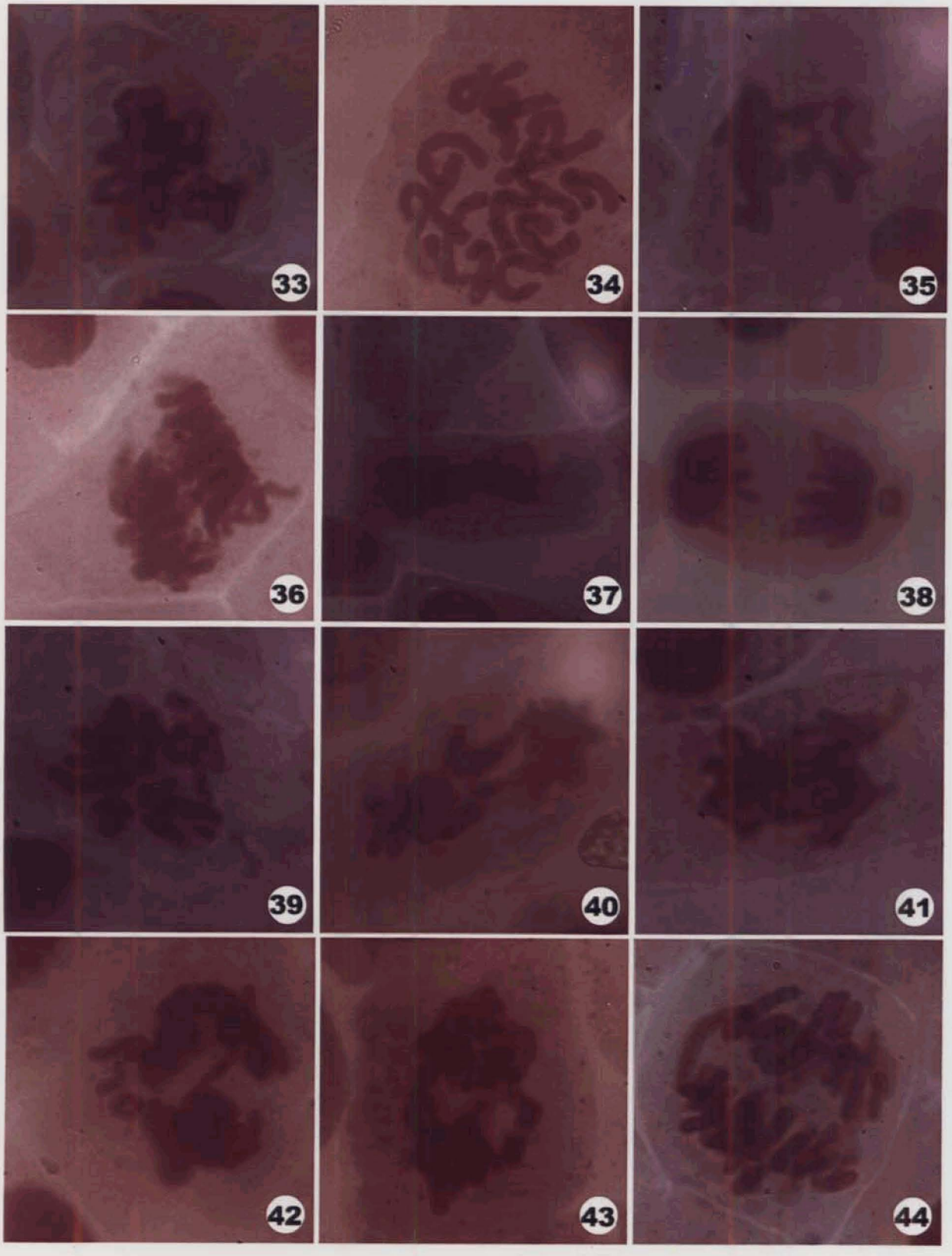


FIGURE LEGEND

45. Anaphase with dislocation of a chromosome.
46. Bridges and early movement at anaphase.
47. Diagonal anaphase with early movement.
48. Anaphase showing early movement.
49. Diagonal anaphase.
50. Anaphase with two chromosome bridges.
51. Diagonal anaphase with bridges.
52. Lagging chromosome fragments and a ring at anaphase.
53. Multipolarity, early movement and a chromosome ring at anaphase.
54. Diagonal sticky anaphase with a single chromosome bridge.
55. Star anaphase.
56. Anaphase erosion.

PLATE - 6

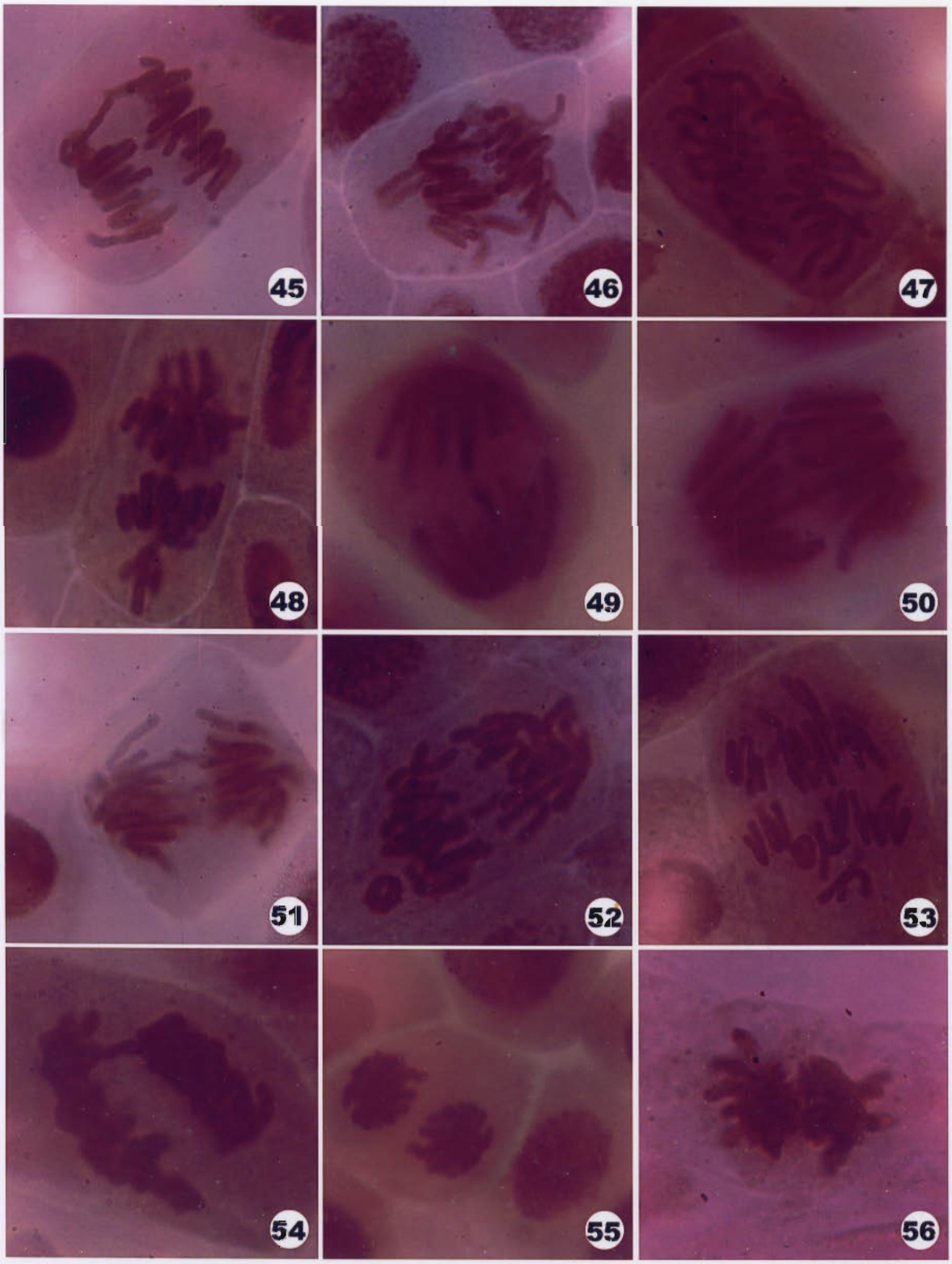


FIGURE LEGEND

57. Stickiness and dissolution of chromosomes during anaphase.
58. Stickiness at telophase.
59. Aberrant telophase with a lagging chromosome.
60. Two parallel bridges at telophase.
61. Diagonal telophase.
62. Multiple bridges at telophase.
63. Non-orientation and stickiness at telophase.
64. Sticky telophase with two micronuclei.
65. Chromosome stickiness with double bridges at early telophase.
66. Telophase with sticky bridge.
67. Sticky telophase with a bridge and a ring chromosome.
68. Polyploid cell.

PLATE - 7

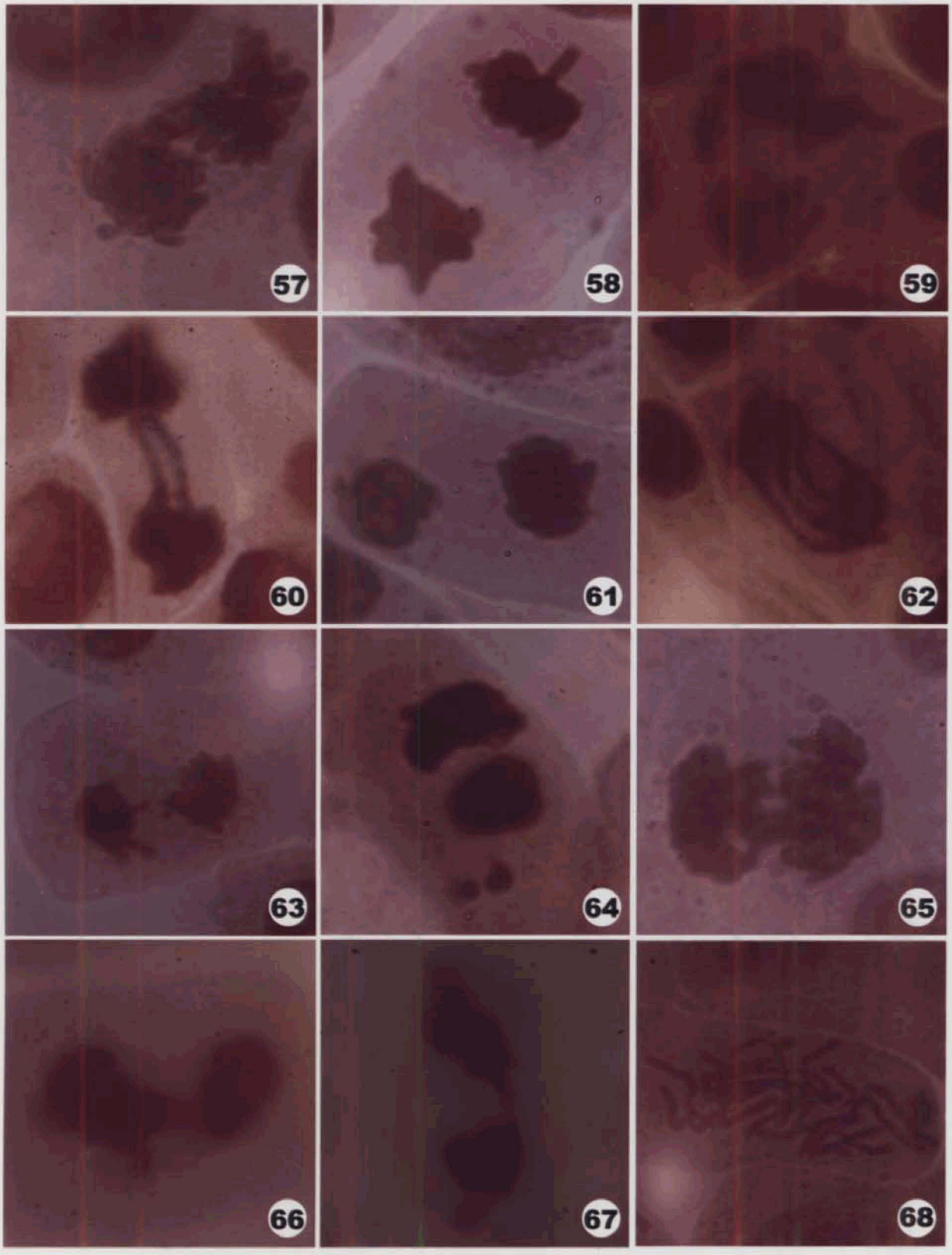


FIGURE LEGEND

Figs. 69 -116. Chromosomal aberrations induced by organophosphorus Pesticides in *Allium sativum* (x 1000).

69. Abnormal prophase.
70. Nuclear lesion.
71. Interphase cell with a micronucleus.
72. Binucleate cell.
73. Binucleate cell with nuclear lesion.
74. Binucleate cell with nuclear extrusion.
75. Nuclear deformation.
76. Ball metaphase.
77. Chromosome stickiness at metaphase.
78. Chromosome clumping at metaphase.
79. Sticky irregular metaphase with chromosome fragments.
80. Diagonal metaphase.

PLATE - 8

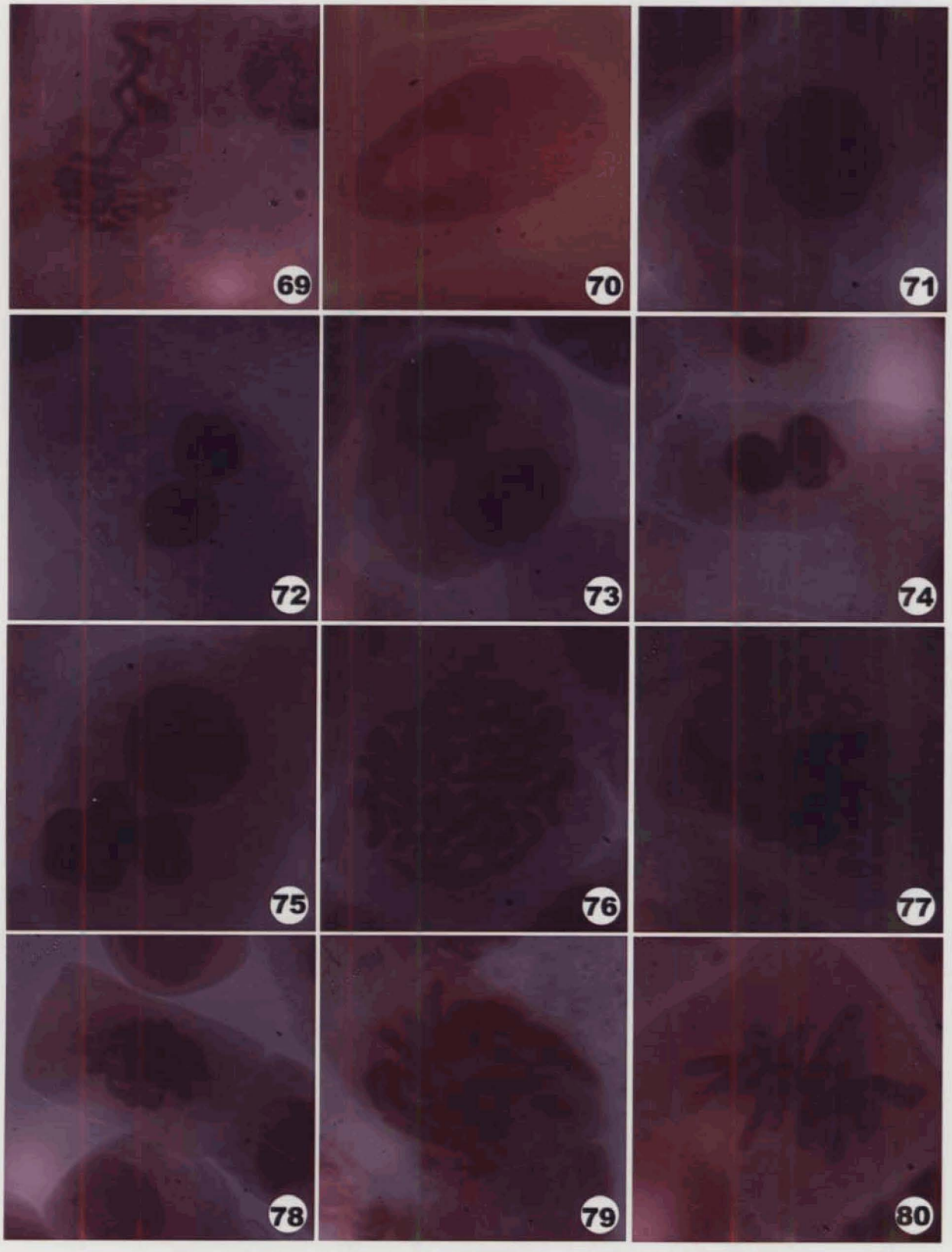


FIGURE LEGEND

81. Sticky metaphase showing chromosome erosion.
82. Sticky metaphase in a hyperploid cell.
83. Oblique metaphase.
84. Misorientation of chromosomes at metaphase.
85. Cells at C-metaphase.
86. Anaphase with lagging chromosomes.
87. Diagonal anaphase with early movement and a dislocation.
88. Anaphase with two ring chromosomes.
89. Anaphase with multiple bridges.
90. Obliquely placed star anaphase.
91. Diagonal anaphase with early movement.
92. Disturbed chromosomes at anaphase.

PLATE - 9

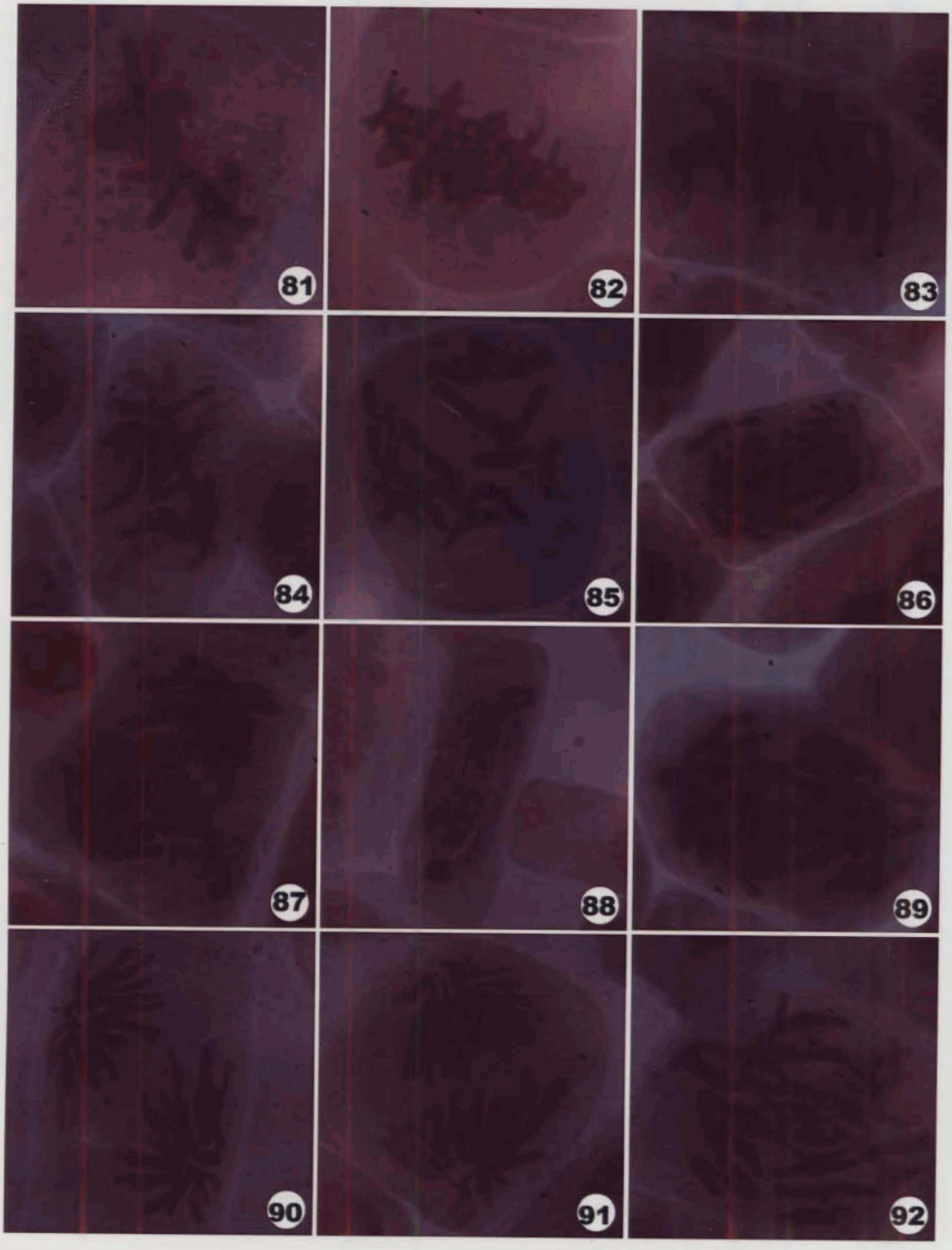


FIGURE LEGEND

93. Diagonal stathmo-anaphase.
94. Diagonal anaphase.
95. Anaphase with early movement and bridge formation.
96. Diagonal sticky anaphase.
97. Multipolarity with chromosome fragments at anaphase.
98. Cell showing unequal segregation of chromosomes.
99. Multipolarity at anaphase.
100. Tripolarity with persistent bridges at late anaphase.
101. Sticky star anaphase.
102. Unequal separation, misorientation and non-synchronous groups of chromosomes at anaphase.
103. Anaphase with bridges and early movement.
104. Stathmo-anaphase.

PLATE - 10

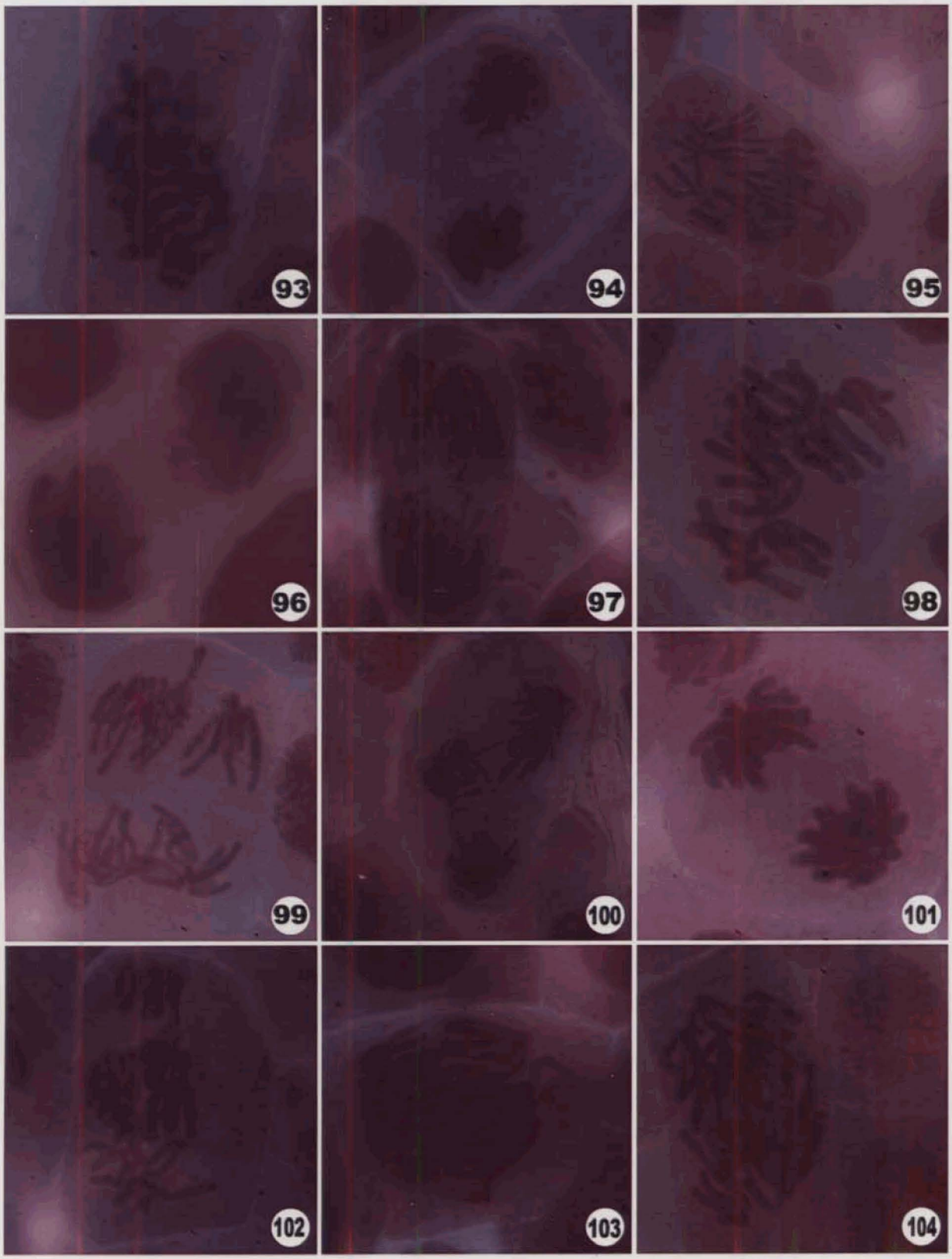
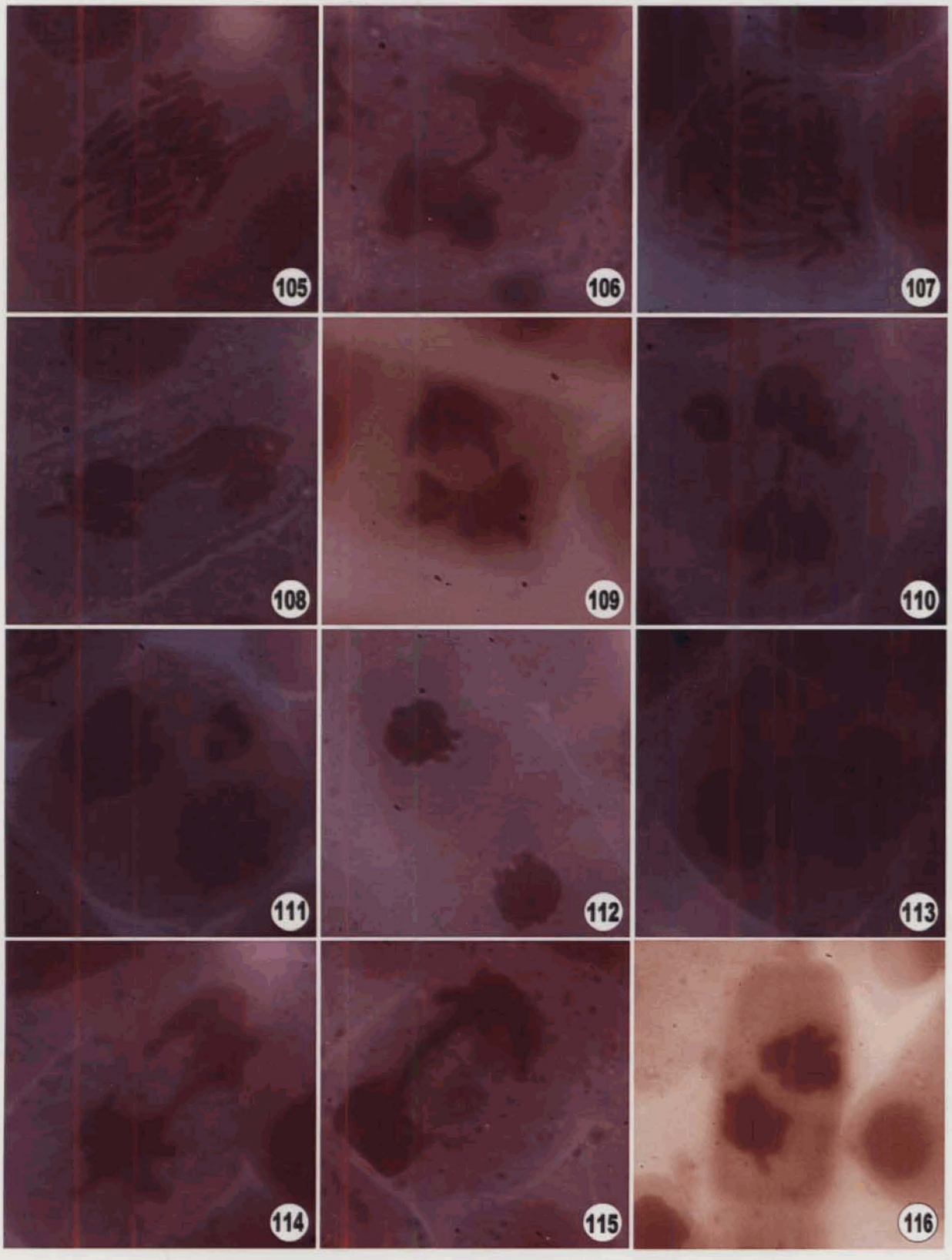


FIGURE LEGEND

105. Chromosome bridges and dislocated chromosomes.
106. Bridge at late anaphase.
107. Non-synchronous movement and scattering of chromosomes at anaphase.
108. Sticky anaphase with chromosome fragment and bridges.
109. Tripolarity and chromosome laggard at anaphase.
110. Sticky bridge and a deserted chromosome.
111. Tripolarity and unequal groups of chromosomes at telophase.
112. Sticky telophase.
113. Diagonal sticky telophase.
114. Chromosome bridge and laggard at telophase.
115. Bridge at telophase.
116. Laggard at telophase.

PLATE - 11



sulphonate (MMS) and dichlorvos (DDVP) on *E. coli*. In all the tests, both compounds showed similar pattern of activity and the results are consistent with their known ability to alkylate DNA. Wild (1973) investigated that microbial mutation test system cannot be directly extrapolated to man. These test systems can contribute to elucidation of certain basic aspects of chemical mutation induction such as kinetic and types of DNA alteration.

Ashton and Crafts (1973) stated that all herbicides that reduced ATP levels are strong inhibitors of RNA and protein synthesis. The inhibition of DNA, RNA and protein synthesis with 2, 4-dinitrophenol treatments had been noted by using tritium labelled precursor. The effect was more drastic at higher concentrations. Thus the degree in division frequency by the phenolic herbicides has some correlation with the inhibition of DNA synthesis. This inhibition in DNA, RNA and protein synthesis by DNP might be due to the reduction of oxidative phosphorylation in plants resulting in the lowering of ATP levels.

Mohan (1973) observed that most chemical mutagens are known to induce several types of DNA alterations with different efficiencies, the 5-MTR system was chosen to compare the over all mutagenic activity of three reference mutagens, β-PL, MNNG and MMS and of eight organophosphates widely used as insecticides (parathion, methyl parathion, Malathion, dimethoate, diazinon, dichlorovos, methyl oxydemeton and bidrin). All of the tested compounds can be regarded as monofunctional alkylating agents. The concentration dependent mutagenic activity of all mutagenic compounds is linear. Davids (1973); Swietlinska *et al.* (1974) have reported that mitotic inhibition was accompanied by DNA synthesis inhibition.

Ayonoadu (1974) observed that the chromosomal DNA amount is changable to some extent within a species on exposure to organophosphorus chemicals.

Lawley *et al.* (1974) examined that dichlorvos reacted in cells with protein through methylation and phosphorylation. The resulting activity in the protein from treated *E. coli.* cells was 20-30 times higher than that in nucleic acids. The data makes it clear that dichlorvos reacts in cells preferentially with protein. This reaction is partly due to methylation of protein, protein phosphorylation probably contributing as well. According to Araratyan and Azatyan (1974) IAA has been shown to stimulate the beginning of DNA synthesis to provide radiation protection.

Adam (1975) studied that the vital processes of the crop itself may be affected by the use of pesticides. The most serious injury is that on the cytological level, where the DNA molecule and consequently the gene is altered. Regular application of pesticide to crop plants has been cited as a possible source of genetic damage leading to instability.

Hanna and Dyer (1975) tested 140 organophosphorus compounds for mutagenic activity in bacteria. It was found that 20% gave positive mutagenic responses and that these groups of chemicals produce base substitution rather than frame shift mutations. In most cases, the DNA repair genes suffered mutagenic activity.

Fishbein (1976) studied the genetic effects of the organophosphorus insecticides. Momparler and Myronkaron (1976) studied the effect of Adriamycin on cell free system and intact cells of hamster fibro sarcoma. This revealed that Adriamycin is a potent inhibitor of DNA and RNA biosynthesis.

According to Shirasu *et al.* (1976) evaluation of the genetic toxicity of pesticides requires elucidation of different aspects such as (1) physical and biological accumulation or degradation of chemicals in the environment (2) metabolism in human beings and (3) genetic relativities with cellular components such as DNA causing point mutations and/or chromosome aberrations.

Evans (1977) pointed out that DNA molecule is responsible for the linear continuity of the chromosomes and the chromosome breakage is due to mis-repair of DNA. Rost (1977) discovered that the mitotic activity depends on the availability of ATP, the cell division again indirectly influenced by pesticidal substances, which inhibit ATP formation. Therefore pesticides produce phytotoxicity by acting directly, aware of the steps in the reactions that constitute nucleic acid and protein synthesis or indirectly by inhibiting cellular energy production that is required to drive the biosynthetic reaction.

Presence of pesticide molecules or their derivatives may disturb the synthesis or the structure of DNA or may cause inhibition of cell's entry into mitosis (Kihlman, 1966) either by delay in the onset of G_2 (Gelfant, 1963; Evans and Scott, 1964; Wacchter and Baserga, 1982) and/or delay in the S-phase (Mitchison and Creanor, 1971). Arrest of division would take place in most of these eventualities.

According to Pushpangadan *et al.* (1979) parthenin treatment damaged the meristematic cells of roots. The damage increased with increased parthenin concentration. The nuclear membrane and spindle fibres were dissolved and there was inhibition of protein formation in the chromosomes and finally of DNA itself. The disintegration of the nuclei starts with vacuolization, which increases, bursts and destroys all the cellular material. Whereas cells in which protein formation alone is affected are able to recover after removal of parthenin in cells. Where DNA has been damaged with higher parthenin concentrations, recovery is impossible.

Mercykutty and Stephen (1980) proved that Adriamycin inhibits not only DNA synthesis but also protein synthesis, either directly or indirectly by its binding with DNA resulting in the prevention of its unwinding for transcription

of spindle protein messengers. To arrest cell division, the best time of application appears to be the G_1 just before the onset of DNA synthesis.

Chand and Roy (1981) studied the effect of 2, 4-dinitrophenol (DNP) on *Nigella sativa*, to note the changes in mitosis, DNA, RNA and protein synthesis. The chemical affected division frequency considerably and chromosomal abnormalities like sticky bridge, fragmentation, micronuclei etc. were recorded. By using precursors of nucleic acid and protein synthesis, it was found that DNP also inhibited DNA, RNA and protein synthesis. The decrease in division frequency can be correlated with DNA synthesis.

Hess (1983) revealed that, the metabolic disturbances due to the effect of herbicidal chemicals generally lead to the inhibition of cell division which in turn is believed to be due to the effect on the synthesis of DNA, RNA, protein and also showed turbogenic action of isoproturon in plant cells at the treatment time. Several other pesticides had also been reported to induce abnormalities by affecting mitotic spindles and are generally considered insignificant from the mutation point of view unless polyploid or aneuploid cells are induced (Onfelt and Klastersk, 1983).

The reduction in buffer soluble protein and total free amino acid indicates that Monocrotophos interferes with nitrogen metabolism as observed by Rost and Reynold (1985) in *Pisum sativum*.

Pandita (1986) proved the mutagenic studies on the insecticide Metasystox-R with *Allium cepa*. The chromosome toxicity was found increasing with the increase in the insecticide concentration. Pooling of chromosomes suggest that the insecticide may be stimulating some of the lyases involved in protein removal from DNA. When the proteins are removed from DNA, nucleases have an access to act upon it. Endonucleases cause internal cuts in DNA, thus leading to the formation of fragments. Endonucleases act upon the

terminal nucleotides and degrade the DNA. Both the phenomena seem to be in operation as chromosome fragments as well as decrease in 2C DNA content was observed after the insecticide exposure.

Rao *et al.* (1987) suggested that both increase and decrease in DNA content may be associated with evolution. Nitrogen compounds play essential roles in plant metabolism being primary product of inorganic nitrogen assimilation and precursors of protein and nucleic acids.

RNA content may be directly related to the DNA content of the cell. Ignacimuthu and Babu (1988) studied nuclear DNA and RNA amounts in the wild and cultivated urd and mung beans and their M_1 plants, pointed out that the decrease in mean DNA content per cell is directly proportional to the decrease in the rate of RNA turn over. Such an induced decrease in RNA synthesis has been reported in irradiated seeds of lettuce also (Yearly and Stone, 1975). Yonus *et al.* (1988) studied that the systemic insecticide Nuvacron, induced tetraploids and found that the disturbed chromosomes, may be due to the effect on spindle fibre in addition to the effect on DNA.

Adam *et al.* (1990) observed the alteration in nucleic acids, protein content and mitotic division of *Vicia faba* root tip cells after the treatment with Malathion and Tamaron insecticides.

Kumar and Sinha (1991) studied the genotoxic effects of two pesticides (Rogor and Bavistin) and an antibiotic (Streptomycin) in meiotic cells of grass pea (*Lathyrus sativus* L.). The deleterious effects produced by them were found to be threatening seriously the genetic hygiene of living beings including man.

George and Ghareeb (2001) examined the insecticide Cyolan for the induction of mitotic and meiotic abnormalities and changes in the banding patterns of M_2 seed protein in *Vicia faba* plants. The used insecticide solution had mitodepressive effect and induced a wide range of mitotic and meiotic

abnormalities. On the protein level, the results for the 3 major fractions of seed storage proteins in *Vicia faba*, as revealed by SDS-polyacrylamide gel electrophoresis, showed mutagenic effects by the insecticide. In general, all treatments caused decrease in the amount of protein.

MATERIALS AND METHODS

Kochuthressia M. V. "Cytotoxic and biochemical effects of some organophosphorus pesticides in Allium cepa L. and Allium sativum L." Thesis. Department of Botany, University of Calicut, 2005

MATERIALS AND METHODS

Test materials: -

The test materials used for the cytotoxic and biochemical assays are as follows:

1. *Allium cepa* (onion) 2n = 16 and
2. *Allium sativum* (garlic) 2n = 16.

Healthy bulbs of *A. cepa* and *A. sativum* were collected and the bulbs were planted in pure sandy soil and watered adequately. The healthy young roots of fresh bulbs of both are used as a classical test material for cytotoxic studies. Because of the following reasons, these two materials *Allium cepa* and *Allium sativum* were selected as test organisms for this investigation.

- They can be easily propagated.
- They need a very short space for cultivation.
- They are easy to handle.
- They produce a large number of roots within a short period.
- They possess large sized cells with small number of chromosomes and the chromosomes are large enough for easy cytological observations.
- The chromosomes show less variation in size and shape.
- The root tip meristem possesses a large number of actively dividing cells.
- It can express the symptoms of clastogenicity and turbogenicity very easily according to the changing environments.
- Feasibility to squash.

Fresh healthy and certified bulbs of *Allium cepa* and *Allium sativum* were obtained from Agricultural University, Coimbatore and the five organophosphorus insecticides were purchased from Agro chemicals, Thrissur.

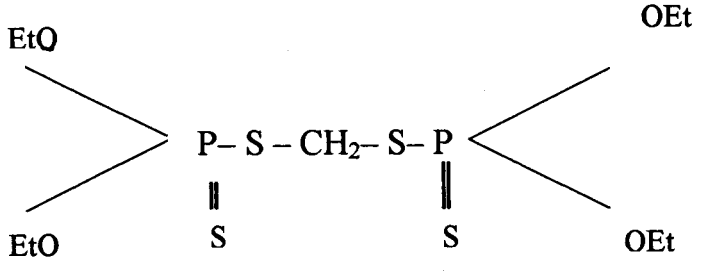
Insecticides used for the treatment: -

The insecticides used for the treatment were Ethion, Malathion, Metacid, Nuvan and Rogor. All these chemicals belong to the same chemical family of organophosphorus pesticides.

Ethion: -

Food Machinery and Chemical Corporation of USA developed Ethion in 1956. It was synthesized by the reaction of diethyl phosphorodithioic acid with bromochloromethane or dibromomethane in the presence of alkali. The product was a yellow liquid. Ethion is practically insoluble in water and highly soluble in aromatic hydrocarbons (alcohol, acetone, benzene, chloroform etc). It is slightly soluble in mineral oil (Petroleum 5%). It gets slowly oxidized on exposure to air. It is a non-systemic fast acting insecticide with contact action and is a cholinesterase inhibitor. It is used to control the motile stages of eggs of spider mites in fruit cultivation. It shows secondary action against aphids, codling moth and others. It was used on a broad range of ornamental, fiber and food crops. It was found to be incompatible with alkaline preparations and formulating materials. It is toxic to fish and bees (Hartley and Kidd, 1983).

Chemical Structure



Molecular formula : $C_9H_{22}O_4P_2S_4$

Molecular weight : 384.48

Density : 1.215 – 1.230 at 20^0C

Common name : Ethion

Trade names : Rhodocide, Nialate, Ethanox, Ethiol etc.

Chemical name : 0, 0, 0^1 0^1–tetraethyl S, S^1 methylene di (phosphorodithioate).

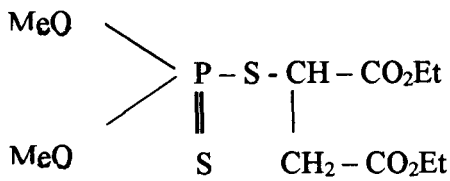
Malathion: -

Malathion was introduced in 1950 by American Cyanamid Company and has been acknowledged as the first organophosphorus insecticide with high selective toxicity. It is synthesized by the addition of dimethyl hydrogen phosphorodithioate to diethyl maleate. Malathion is a wide spectrum non-systemic insecticide and acaricide having brief to moderate persistence and low mammalian toxicity. It is generally non phytotoxic, but may damage cucurbits and various plant species.

It is a colourless or pale brownish yellow liquid. It is soluble in water at 25^0C, miscible with most organic solvents though not in petroleum oils. Hydrolysis is rapid at pH above 7 and below 5. It is incompatible with alkaline pesticides. Heavy metals, particularly iron, catalyse its decomposition.

Malathion is a safe general-purpose insecticide suited for the control of sucking and chewing insects on fruits and vegetables and for the control of mosquitoes and flies. Owing to its low mammalian toxicity and high insecticidal activity, the WHO for the eradication of *Anopheles* mosquitoes uses Malathion on a large scale.

Chemical Structure: -



Molecular formula : $C_{10}H_{19}O_6PS_2$

Molecular weight : 330.36

Density : 1.23 ($4^0C - 25^0C$)

Common name : Malathion

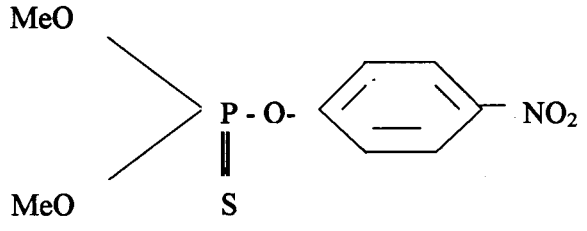
Trade names : Malathion, Cythion, Malmed, Calmathion.

Chemical name : S–1, 2–bis (ethoxy carbonyl) ethyl phosphorodithioate

Metacid (Methyl parathion): -

Bayer A.G introduced Metacid in 1949. It is a white crystalline powder, soluble in most organic solvents eg. alcohol, acetone, benzene and chloroform. It undergoes hydrolysis in alkaline and acidic media and gets isomerized on heating. It is non-corrosive and is soluble in water at 25^0C. It is a cholinesterase inhibitor. It controls biting and sucking insects in the cultivation of vines, fruits, vegetables and field crops. Generally, it is good in crop tolerance. It is compatible with non-alkaline preparations. It is toxic to fish, animals of fish diet and bees. As it belongs to organophosphate group, it inhibits the activity of blood cholinesterase.

Chemical Structure



Molecular formula : $C_8H_{10}NO_5PS$

Molecular weight : 263.21

Density : 1.358 ($4^0C - 20^0C$)

Common name : Methyl parathion

Trade name : Metacid

Chemical name : 0, 0 – dimethyl 0 – 4 – nitrophenyl phosphorothioate

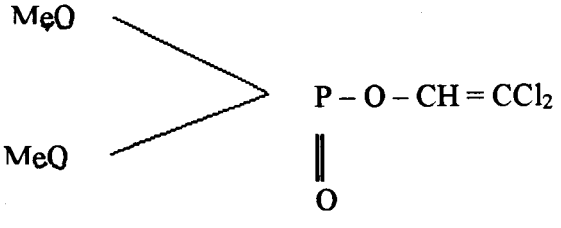
Nuvan (Dichlorvos): -

It was first found as a highly insecticidal impurity of trichlorfon in 1955 (Mattson *et al.*, 1955). The manufacturing is done by the Perkon reaction of trimethyl phosphite and chloral. Dichlorvos is a colourless liquid, soluble in water and miscible with most organic solvents.

Dichlorvos is a short-lived, wide spectrum contact and stomach insecticide with fumigant and penetrating action and low residual activity. It has rapid knock down activity against flies, mosquitoes, moths etc. It is also utilized as an anthelminthic for worms in pigs. It gets rapidly degraded in mammals through the initial splitting of the vinyl or the methyl ester linkage. It is non phytotoxic and is used as a household and public health fumigant especially against mosquitoes and other Diptera, in addition to crop protection uses. It is a colourless liquid, stable to heat, but gets hydrolysed; corrosive to iron and mild steel but non-corrosive to stainless steel and aluminium.

It is effective against flies, often used for glasshouse fumigation and kills most of the glasshouse pests. Also used on out door fruits and vegetables where rapid kill is required, close to harvest. It will kill sap sucking and leaf – mining insects also.

Chemical structure



Molecular formula : $C_4H_7Cl_2O_4P$

Molecular weight : 220.98

Density : 1.415 ($4^0C - 25^0C$)

Common name : Dichlorvos

Trade names : Nogos, Nuvan, Vapona, Atgard, DDVP.

Chemical name : 2, 2-Dichlorovinyl dimethyl phosphate

Rogor (Dimethoate): -

A systemic and contact insecticide and also a cholinesterase inhibitor used mainly against fruit flies and aphids. The pure compound is a white solid, slightly soluble in H_2O, soluble in most organic solvents except saturated hydrocarbons such as hexane. It is stable in aqueous solution but aqueous alkalies readily hydrolyze it. It is incompatible with alkaline pesticides.

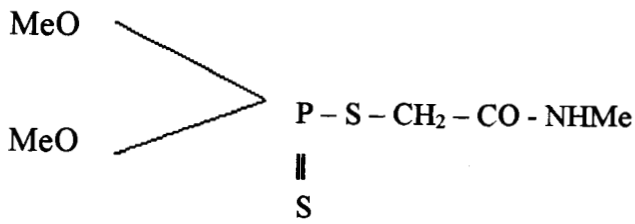
Technical products of Rogor may contain several byproducts. 0, 0, S–trimethyl phosphoro thiolothionate and dimethoate acid methyl ester are the major impurities. The impurities increase the toxicity of dimethoate.

Dimethoate is relatively unstable and decomposes on storage, particularly at elevated temperature and in presence of impurities such as bases and dimethyl phosphorodithioic acid. Polar solvents increase the rate of decomposition. Alkylation reaction is the main degradation mechanism. As iron accelerates the rate of decomposition, iron containers should be avoided for storage.

When sprayed on plants, dimethoate may be oxidized to form the oxygen analog both enzymatically and non-enzymatically. Dimethylation occurs preferably in plants including rice, where as amide cleavage proceeds in cotton to a great extent.

Dimethoate is useful for the control of sucking insects and mites on ornamentals, vegetables, cotton and fruit crops. Also used as a spray for controlling houseflies around farm buildings. It generally has good crop tolerance. It is also toxic to fish and bees.

Chemical structure



Molecular formula : $C_5H_{12}NO_3PS_2$

Molecular weight : 229.28

Density : 1.277 at 65^0C

Common name : Dimethoate

Trade names : Cygon, Dimetate, Rogor

Chemical name : 0, 0-dimethyl S-methylcarbamoylmethyl phosphorodithioate

A. CYTOTOXIC EFFECTS

The healthy and uniform sized certified bulbs of onion and the same conditioned cloves of garlic were selected and washed in distilled water. These selected bulbs were planted in pure sandy soil without manure to prevent other chemically induced chromosomal aberrations. After 2 days, the roots sprouted out in the case of onion, where as, in garlic it took one week.

Determination of time: -

In order to find out the time of peak mitotic activity, the untreated root tips of both *A. cepa* and *A. sativum* were fixed in acetic alcohol (1:3) mixture at different time from 8.30 a.m. to 11.30 a.m. After many trials, it was found that maximum dividing cells (peak mitotic activity) occurred between 9.00 a.m. to 11.00 a.m., under normal sunshine conditions.

Determination of the concentration of pesticides: -

Various concentrations such as 0.0125%, 0.025%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% were prepared by mixing the original pesticide solution

with distilled water and after preliminary analysis, it was confirmed that high concentrations were found to be lethal and thus they were eliminated. Thus the lower concentrations such as 0.0125%, 0.025%, 0.05% and 0.1% were selected for the present study. Control roots were simultaneously treated with distilled water.

Determination of treatment duration: -

Treatment was given in such a way that only the roots were allowed to immerse completely in the test solution. After many trials, using the particular concentrations of five different organophosphorus insecticides, it was confirmed that 1 and 2 hours of treatment produced maximum abnormalities with significant effect on biochemical parameters. When the period of treatment was increased, however, high concentrations were found to be lethal and thus they were also eliminated.

Mode of treatment with insecticide: -

Various concentrations of the insecticides such as Ethion, Malathion, Metacid, Nuvan, and Rogor were prepared by mixing it with different volumes of distilled water. The treatment solutions were poured into different cleaned plastic trays to the brim. Control was also maintained under identical conditions. The mouth of the plastic trays were covered with thick plastic board containing a number of holes, good enough to fit onion bulbs so that only the roots of the bulbs got immersed into the treatment solutions. Bulbs with 1 or 2 cm long roots were ideal for treatment.

Fixation of treated roots: -

The roots of both plants after treatment for different time durations were excised, thoroughly washed in distilled water to remove the traces of the pesticides left on the surface of the roots. These were fixed in acetic acid and ethyl alcohol (1:3) mixture for 3 hours. The root tips containing the meristematic

tissues were excised and they were transferred to bottles containing 70% ethyl alcohol and stored in the refrigerator (10-14^{0}C) until further use.

Preparation of stain: -

This was done according to the procedure of Sharma and Sharma (1990). The stain was prepared as follows. At first, 45ml of glacial acetic acid was mixed with 55ml of distilled water (45% acetic acid). It was poured into a reflex condenser and allowed to boil for 30 minutes. When the mixture started boiling, 2 grams of orcein powder was added little by little and stirred well with a glass rod. When the powder was well dissolved in the solution, it was cooled and filtered into an amber coloured bottle and stored in a refrigerator.

Maceration

Before staining, the root tips were treated with 1N HCl and it was heated for 2 minutes at 60^{0}C for hydrolysis of the material. After that they were washed thoroughly with distilled water. This facilitates the maceration of cell wall, so that while squashing, a clear separation of cells occurred.

Squash preparation

Mitotic squash preparations were made with the help of improved techniques of Sharma and Sharma (1990). The hydrolyzed roots of both plant materials after treatment in different concentrations of different insecticides were stained with 2% aceto orcein for 3 hours. Later these root tips were de-stained with 45% acetic acid. Each root tip was mounted on a drop of 45% acetic acid and squashed with a round cover slip. For better separation of cells, pressure was gently given over the cover slip. Care was taken to avoid debris and air bubbles entering into the cover slip. Slides were sealed with nail polish for temporary storage before observation. 10 slides were prepared in each treatment on both test materials in two different time durations. Each slide thus prepared was observed under the LEICA GALEN III microscope and scanned for the number, types and

kinds of abnormalities. Photomicrographs were taken with a Pentax camera system attached to the microscope. The mitotic index and percentage of abnormalities can be calculated by using the formulas given below:

$$\text{Mitotic index} = \frac{\text{Total no. of dividing cells}}{\text{Total no. of cells observed}} \times 100$$

$$\text{Percentage of abnormalities} = \frac{\text{Total no. of abnormal cells}}{\text{Total no. of cells observed}} \times 100$$

B. BIOCHEMICAL EFFECTS

1. EXTRACTION AND ESTIMATION OF NUCLEIC ACIDS: -

Extraction:

The extraction and separation of nucleic acids were done according to the methods proposed by Schmidt and Thannhauser (1945) and modified by Munro and Fleck (1966). According to this method, the tissues after the desired period of insecticide treatment were, washed thoroughly and fixed in acetic-alcohol (1:3) mixture for 3 hours. After that they were washed in distilled water and wiped with tissue paper and the extreme tips containing the meristematic tissues of each roots were cut and 100 mg of tissue was weighed and freezed overnight. The frozen samples were homogenized in a chilled mortar and pestle with 2 ml. of cooled distilled water. Equal volume of chilled 0.4 N perchloric acid was added to the homogenate so that concentration of perchloric acid was reduced to 0.2 N. The suspension was centrifuged at 6000 rpm. for 15 minutes at room temperature. The supernatant was discarded, which contained acid soluble low molecular weight phosphorus compounds. Sediment was treated with 2ml. of 100% chilled ethanol at room temperature and centrifuged thrice at 6000 rpm. for

15 minutes. The supernatant was discarded each time. Afterwards the residue was washed once with ethanol and ether in the ratio of 3:1 and centrifuged as usual and the supernatant was discarded. The residue was finally washed thrice with diethyl ether, to remove the lipids. At last the residue was air dried in room temperature for 20 minutes. One ml. of 1N KOH was added to the powdery digest, and kept at 37 ^{0} C in an oven for 15 hours. The alkaline digest was cooled at 0 ^{0}C for 1 hour and 1 ml of 0.4 N perchloric acid was added to the suspension; then the concentration of perchloric acid became 0.2 N, which precipitated DNA. The digest was centrifuged and was collected which contain RNA to estimate. The residue was washed again with chilled 0.2 N perchloric acid. These washings were also collected along with the original supernatants containing RNA and the volume of the supernatants was made up to a known volume. Residue was suspended in 1 ml of 0.3N KOH for 10 minutes at room temperature, and the supernatant obtained after centrifugation contained DNA. The residues were re-extracted with 1 ml of 0.3 N KOH and centrifuged again. The supernatant, which contain the DNA, was collected and made up to a known volume of 2 ml.

Estimation of Nucleic acids: -

Estimation of DNA concentration was done according to Burton (1968) and RNA after Markham (1955).

Estimation of DNA: -

It was done according to Burton (1968). The reaction between deoxyribose and diphenylamine is the most frequently used colour reaction for the determination of DNA.

Reagents required: -

- Aqueous acetaldehyde (1.6%): - One ml. of cooled acetaldehyde was transferred by using a cooled pipette into 50 ml of distilled water.

- Diphenylamine reagent: - 1.5 grams of diphenylamine was dissolved in 100 ml.of glacial acetic acid, and 1.5 ml. of concentrated sulphuric acid was then added. Just before use, 0.1ml. of 1.6 % aqueous acetaldehyde was added per 20 ml.of the above reagent.
- Standard solution: -3 mg. of Calf thymus DNA was dissolved in 10 ml of 5 mM NaOH and used as stock standard. Working standards were prepared from this by mixing a measured volume of the stock standard with an equal volume of 1N perchloric acid and heated at 70^0C for 15 minutes. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1ml.of the working standards were pipetted out into a series of test tubes. Duplicates were also taken separately. The volumes were made up to 1ml. in all the test tubes with distilled water. 1ml.of each treated sample extracts were also prepared along with the standard. A test tube with 1ml. of 0.5N perchloric acid was served as a blank. 2ml. of diphenylamine reagent was added to each tube including the blank. All the tubes were incubated for 15hours at 25-30^0C and the absorbance was measured at 600 nm using Bausch & Lomb model spectrophotometer. The standard graph was prepared and the amount of DNA present in the samples was calculated.

Estimation of RNA: -

It was done according to Markham (1955). The principle of this method was that pentoses react with HCl in the hot to give furfural. The latter reacts with orcinol in the presence of ferric ions to give a brilliant green colour, which was due to the function of the pentose concentration.

Reagents required: -

- 1% Orcinol: - It was prepared by dissolving 1g. of orcinol in 100 ml. of distilled water.
- Concentrated HCl.

- 10% $FeCl_3.6H_20$: - 10 grams of ferric chloride was dissolved in 100ml. of distilled water.
- For estimation of riboses, the reagents were prepared by mixing 10ml. of 1% orcinol with 40ml.of concentrated HCl and 1ml. of freshly prepared 10% ferric chloride solution was added to this.
- Standard RNA: - Yeast RNA was used as the standard.

Preparation of standard graph: -

2.5 mg. of yeast RNA was dissolved in 10 ml. of distilled water to prepare the standard stock. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1ml.of the standard solutions was pipetted out into a series of test tubes as working standards. Duplicates were also taken separately. The volumes were made up to 1ml in all the test tubes with distilled water. 1ml.of each treated sample extracts were also prepared along with the standard. A test tube with 1ml. of distilled water was served as a blank. 2ml. of the reagent was added in each tube including blank. The test tubes were closed with marbles and heated for 8 minutes on a water bath at 100^0C and cooled. These have the effect of suppressing the ionisation of the ferric ions and made the colour bluish green. Each solution was red on a Bausch & Lomb model spectrophotometer at 660 nm. The standard graph was prepared and the amount of RNA present in the samples was calculated.

2. ESTIMATION OF BUFFER SOLUBLE PROTEIN: -

It was photometrically determined by folin phenol reagent as reported by Lowry *et al.* (1951).

Reagents required: -

- 0.2M. phosphate buffer (pH. 7.2)
- 10% ice cold trichloro acetic acid.
- 2% ice cold trichloro acetic acid.

- 100% acetone.
- 80% acetone
- 0.1N NaOH.
- 2% alkaline sodium carbonate: - 2grams of Na_2CO_3 was dissolved in 0.1 N NaOH and made up to 100 ml by using the same solution.
- $CuSO_4$-Potassium-Sodium tartrate reagent: - It was prepared by dissolving 1g $CuSO_4$ and 2gms of potassium sodium tartrate separately in 100 ml of distilled water. Equal volumes of the above solutions were mixed together, which will give 0.5% of $CuSO_4$ in 1% of potassium sodium tartrate reagent.
- Alkaline solution: - It was prepared freshly each time prior to use, by mixing 50 ml of alkaline Na_2CO_3 and 1 ml of copper sulphate-sodium potassium tartrate solution.
- Folin-Ciocalteau Reagent: - This commercial reagent was diluted with distilled water in the ratio of 1:1 just before use.
- Standard protein: - Bovine serum albumin was used as the standard. 50 mg of bovine serum albumin was weighed accurately and dissolved in distilled water and made up to 50 ml in a standard flask as standard stock. Working standards were prepared by diluting 10 ml of standard stock solution to 50 ml with distilled water in a standard flask. 1 ml of this solution contains 200 μg of protein.
- Phosphate buffer: - It was prepared by dissolving 2.78 grams of monobasic sodium phosphate and 5.365 grams of dibasic sodium phosphate separately in 100 ml of distilled water. 28 ml of monobasic sodium phosphate solution was mixed with 72 ml of dibasic sodium phosphate solution. This was diluted to 200 ml. with distilled water. pH was adjusted to 7.2 with the pH meter.

FIG. 117. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON MITOTIC INDEX IN ROOT TIP CELLS OF *ALLIUM CEPA* AFTER 1 HOUR TREATMENT

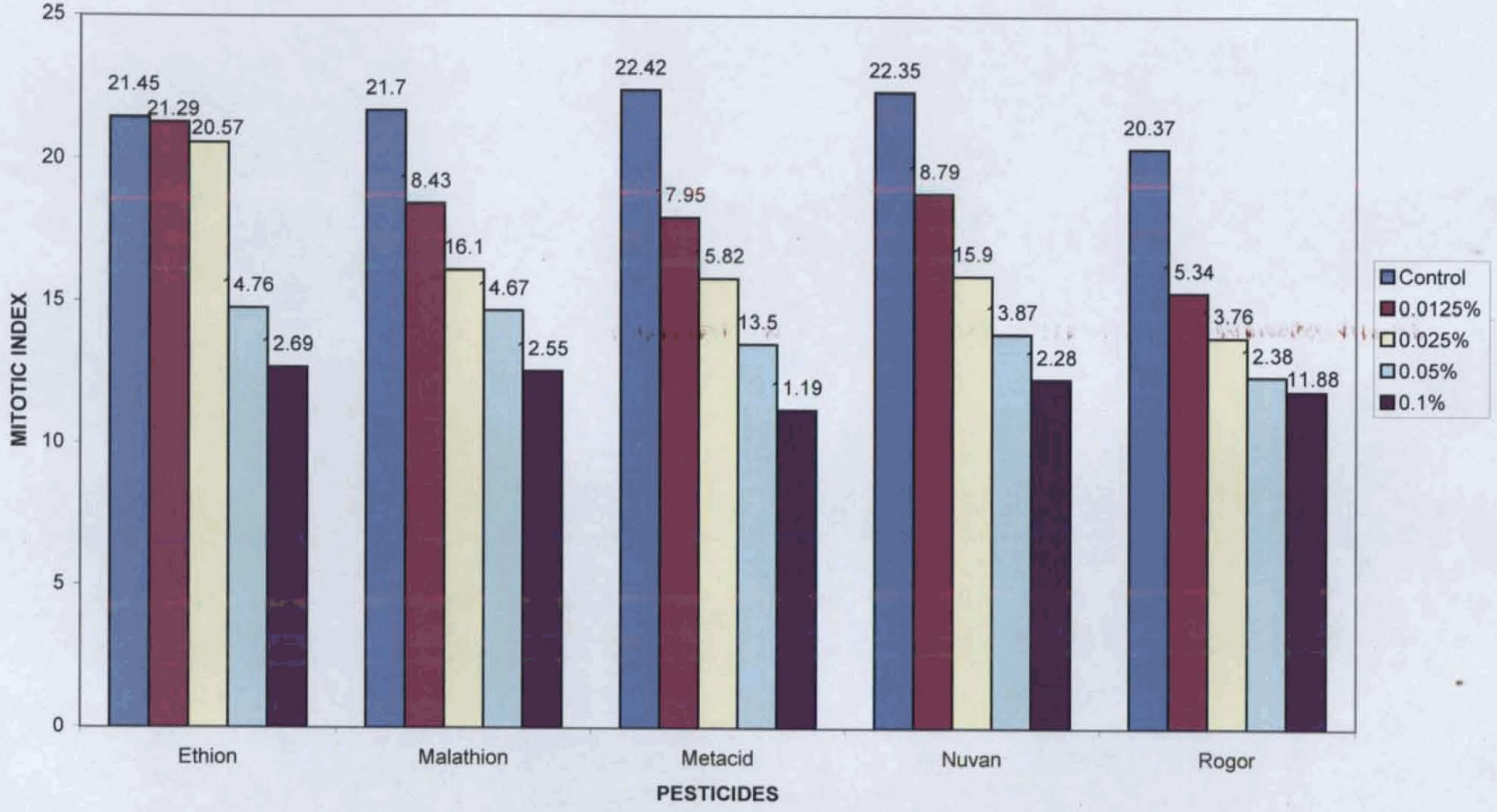


FIG. 118. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON MITOTIC INDEX IN ROOT TIP CELLS OF *ALLIUM CEPA* AFTER 2 HOURS OF TREATMENT

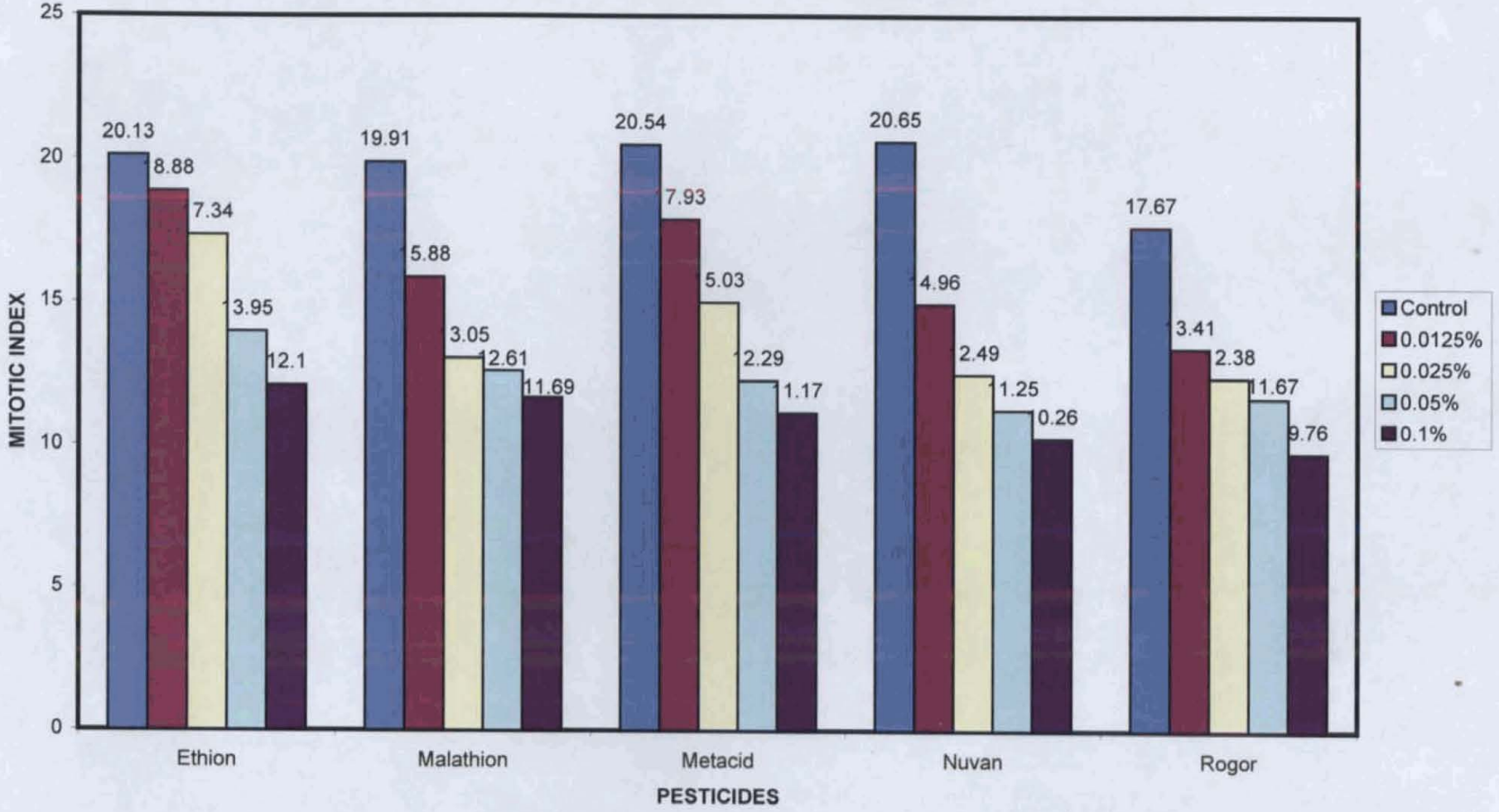


FIG. 119. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON MITOTIC INDEX IN ROOT TIP CELLS OF *ALLIUM SATIVUM* AFTER 1 HOUR TREATMENT

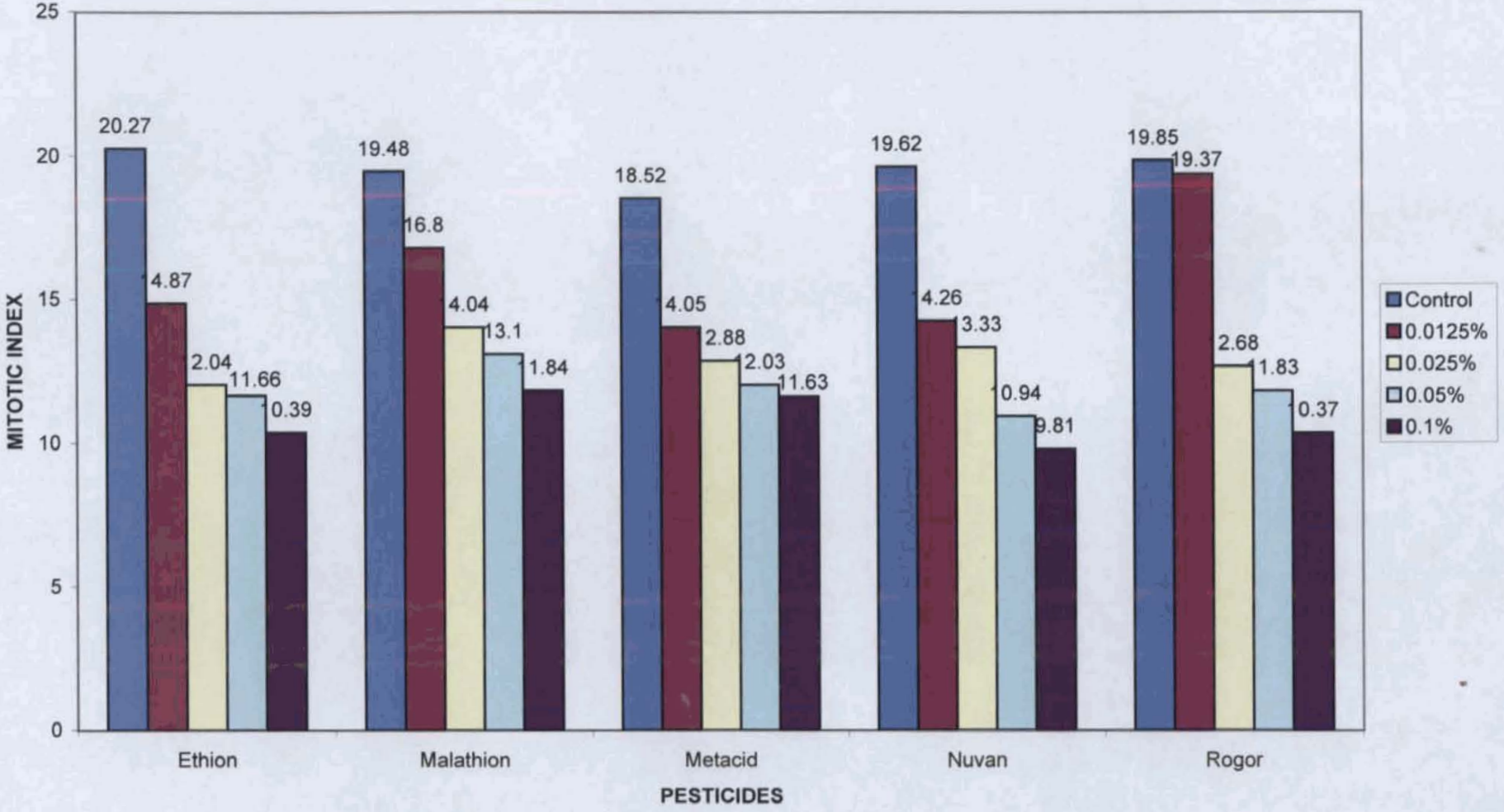


FIG. 120. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON MITOTIC INDEX IN ROOT TIP CELLS OF *ALLIUM SATIVUM* AFTER 2 HOURS OF TREATMENT

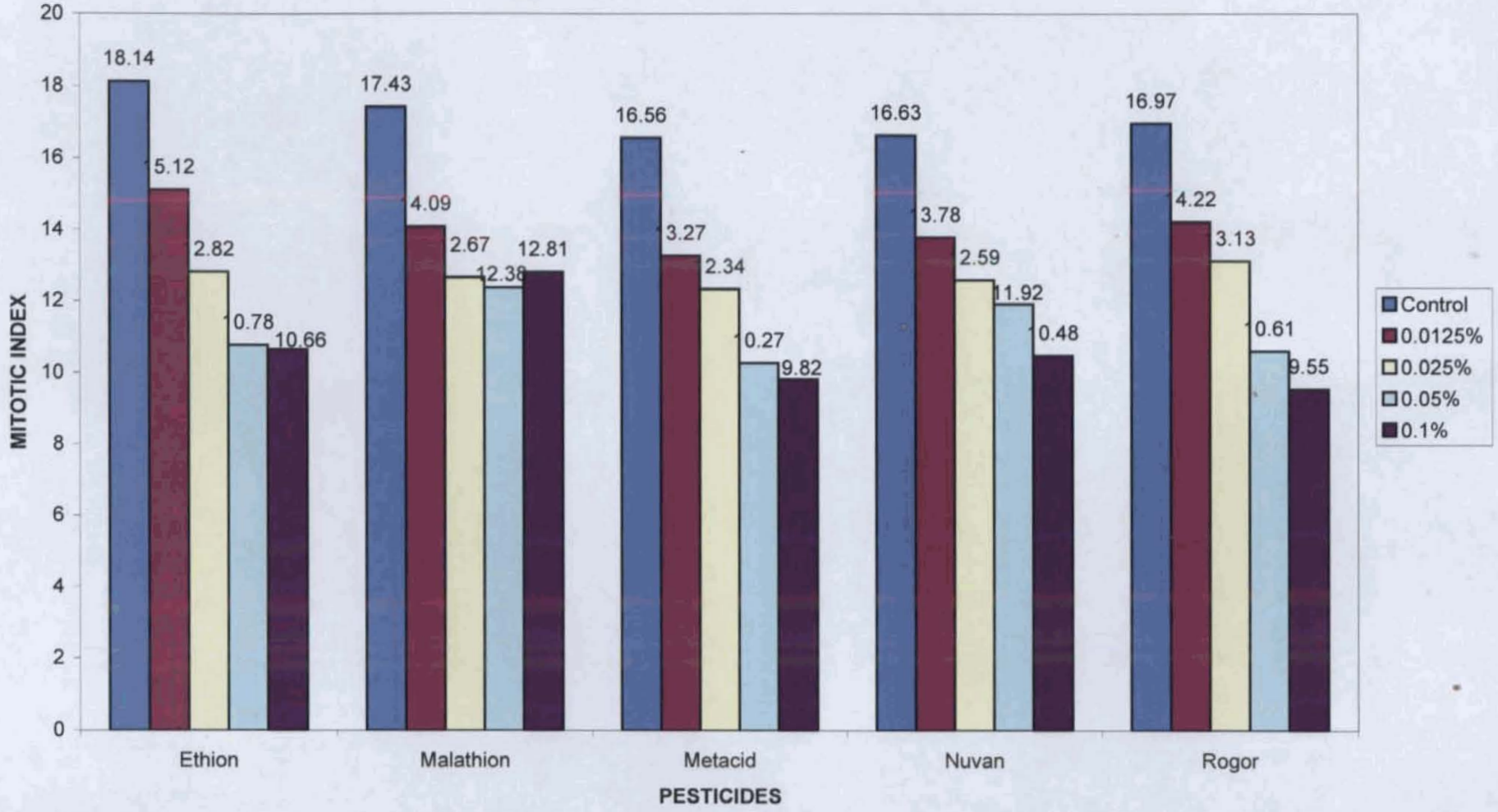


FIG. 121. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON % OF ABNORMAL CELLS IN ROOT TIP CELLS OF *ALLIUM CEPA* AFTER 1 HOUR TREATMENT

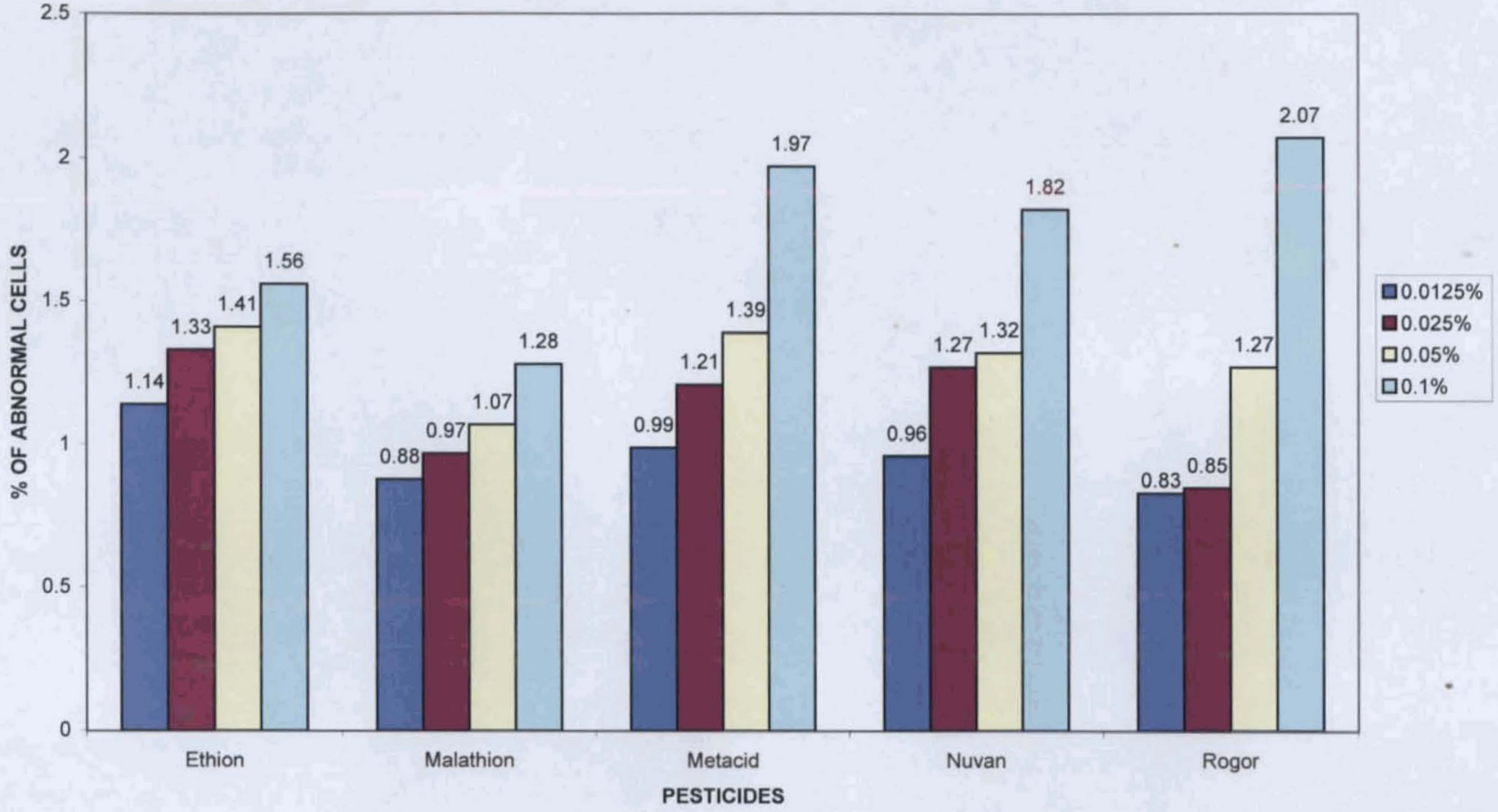


FIG. 122. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON % OF ABNORMAL CELLS IN ROOT TIP CELLS OF *ALLIUM CEPA* AFTER 2 HOURS OF TREATMENT

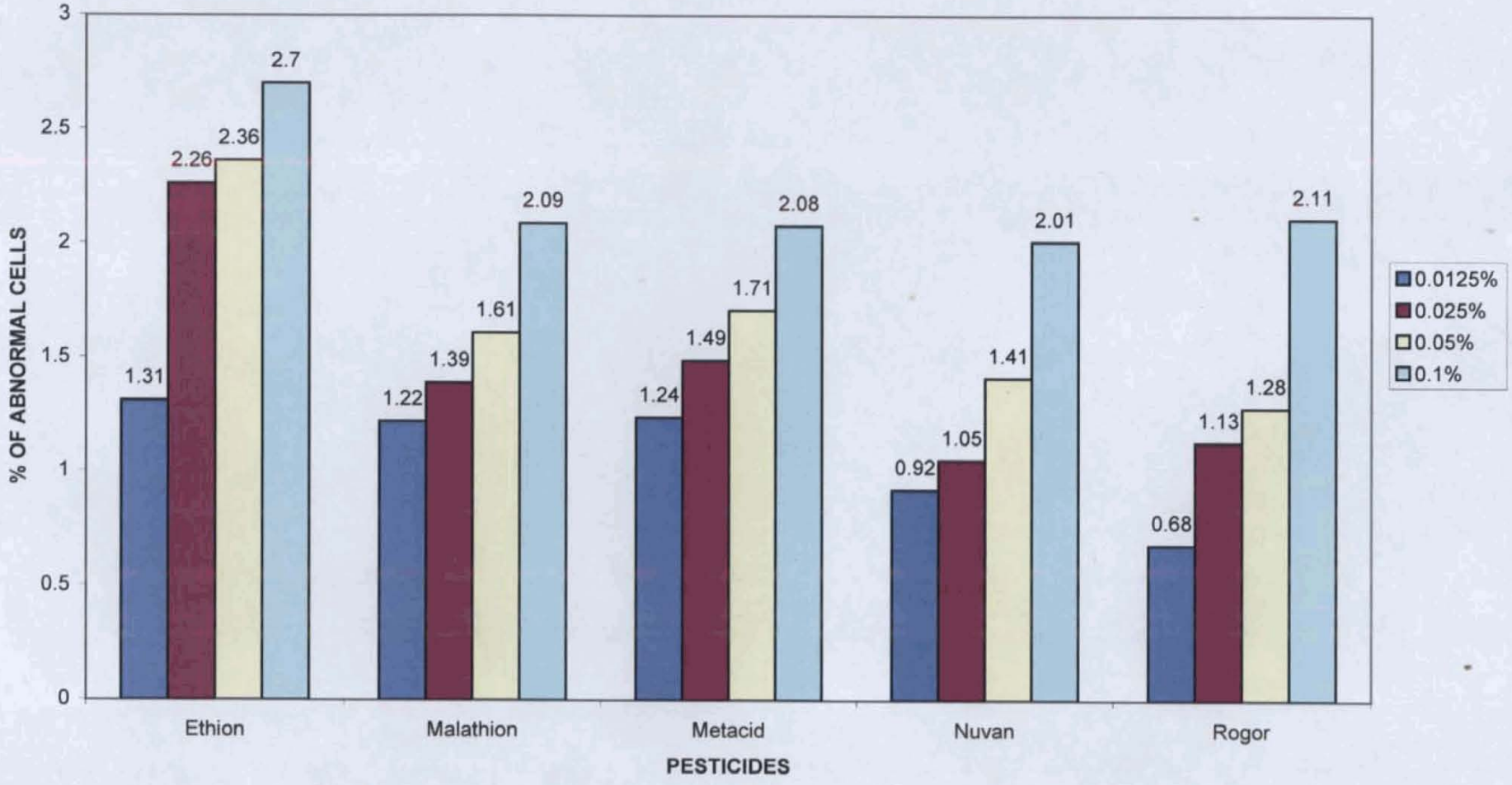


FIG. 123. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON % OF ABNORMAL CELLS IN ROOT TIP CELLS OF *ALLIUM SATIVUM* AFTER 1 HOUR TREATMENT

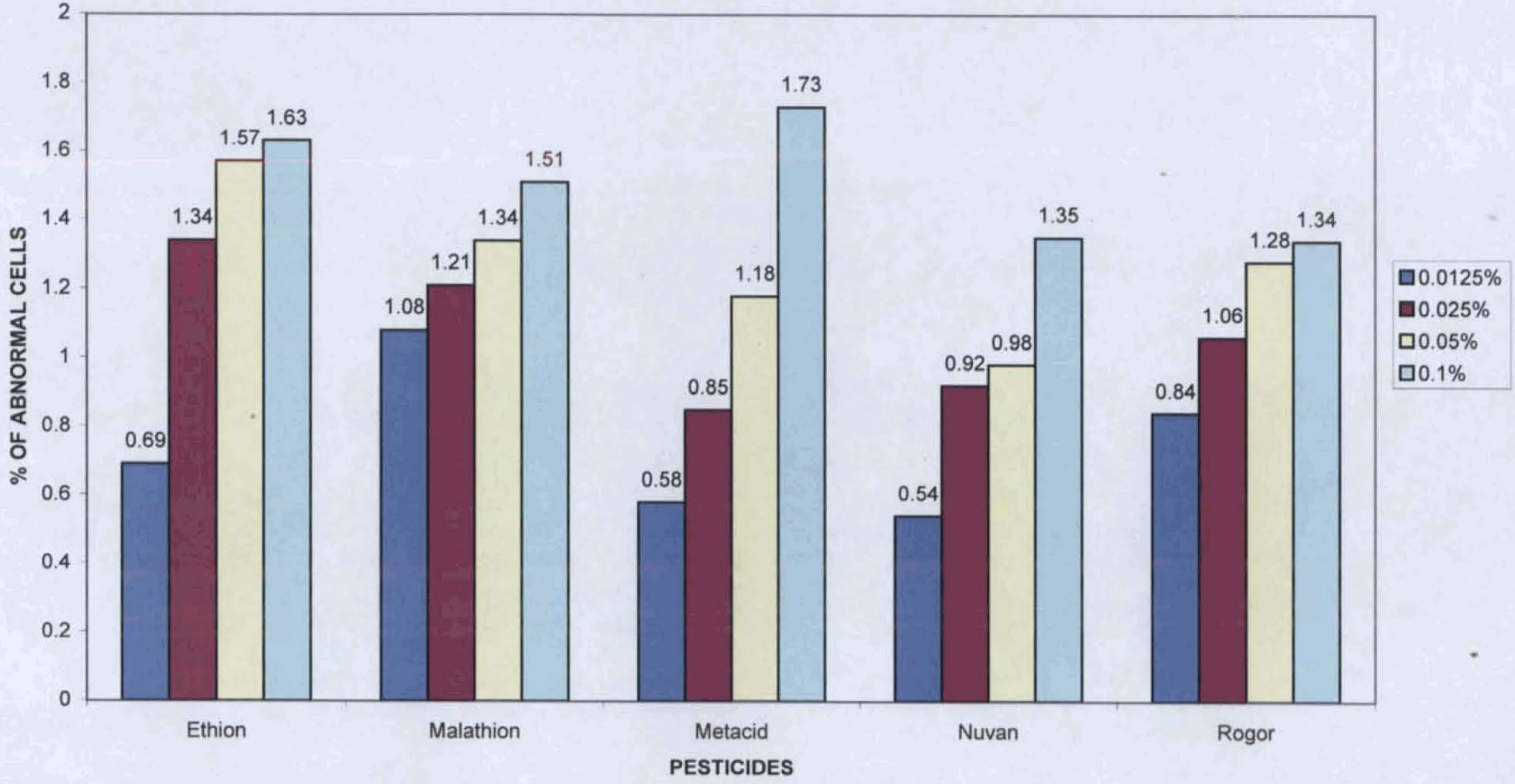


FIG. 124. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON % OF ABNORMAL CELLS IN ROOT TIP CELLS OF *ALLIUM SATIVUM* AFTER 2 HOURS OF TREATMENT

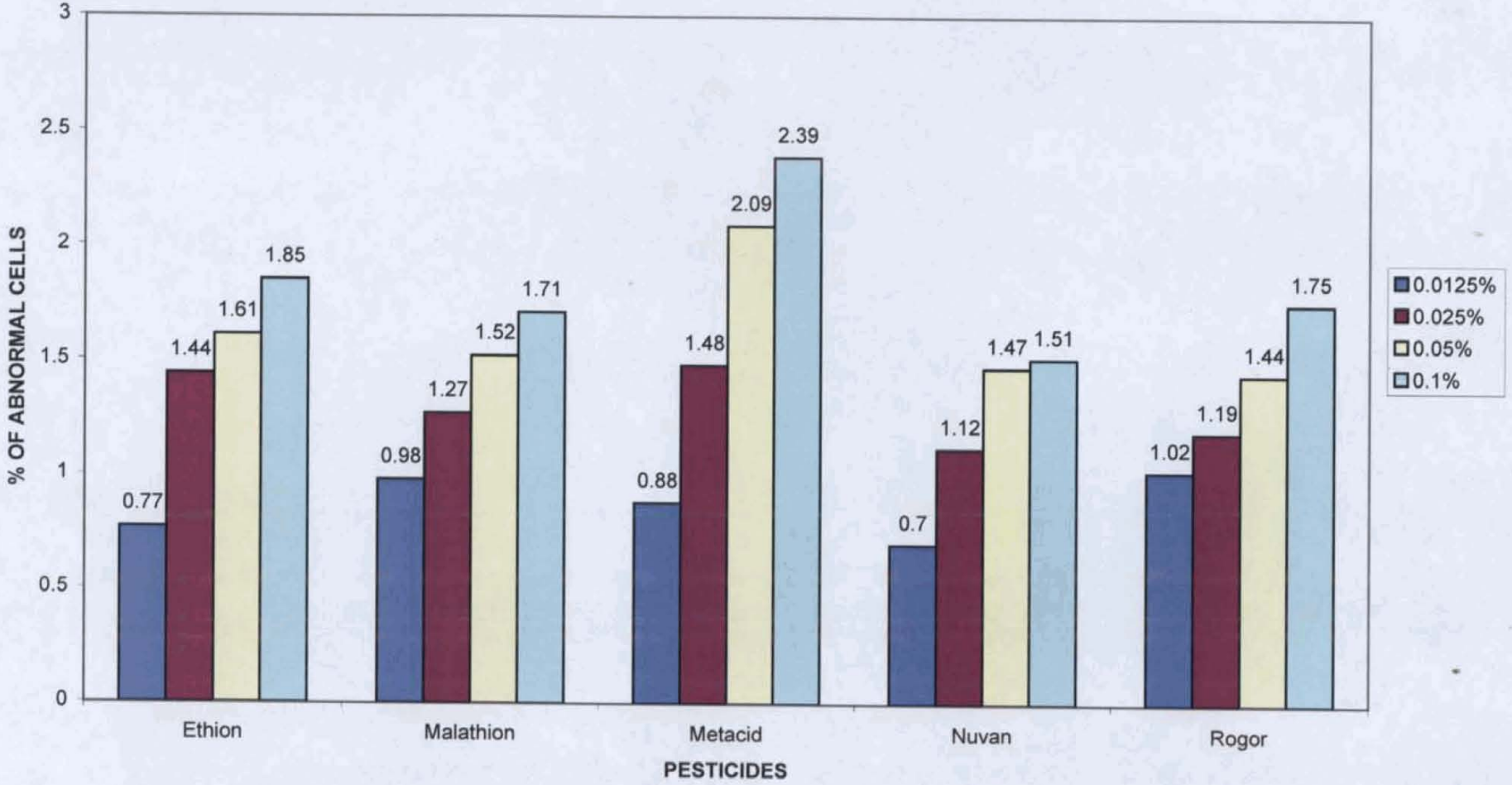


FIG. 125. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON THE AMOUNT OF DNA IN ROOT TIP CELLS OF *ALLIUM CEPA* AFTER 1 HOUR TREATMENT

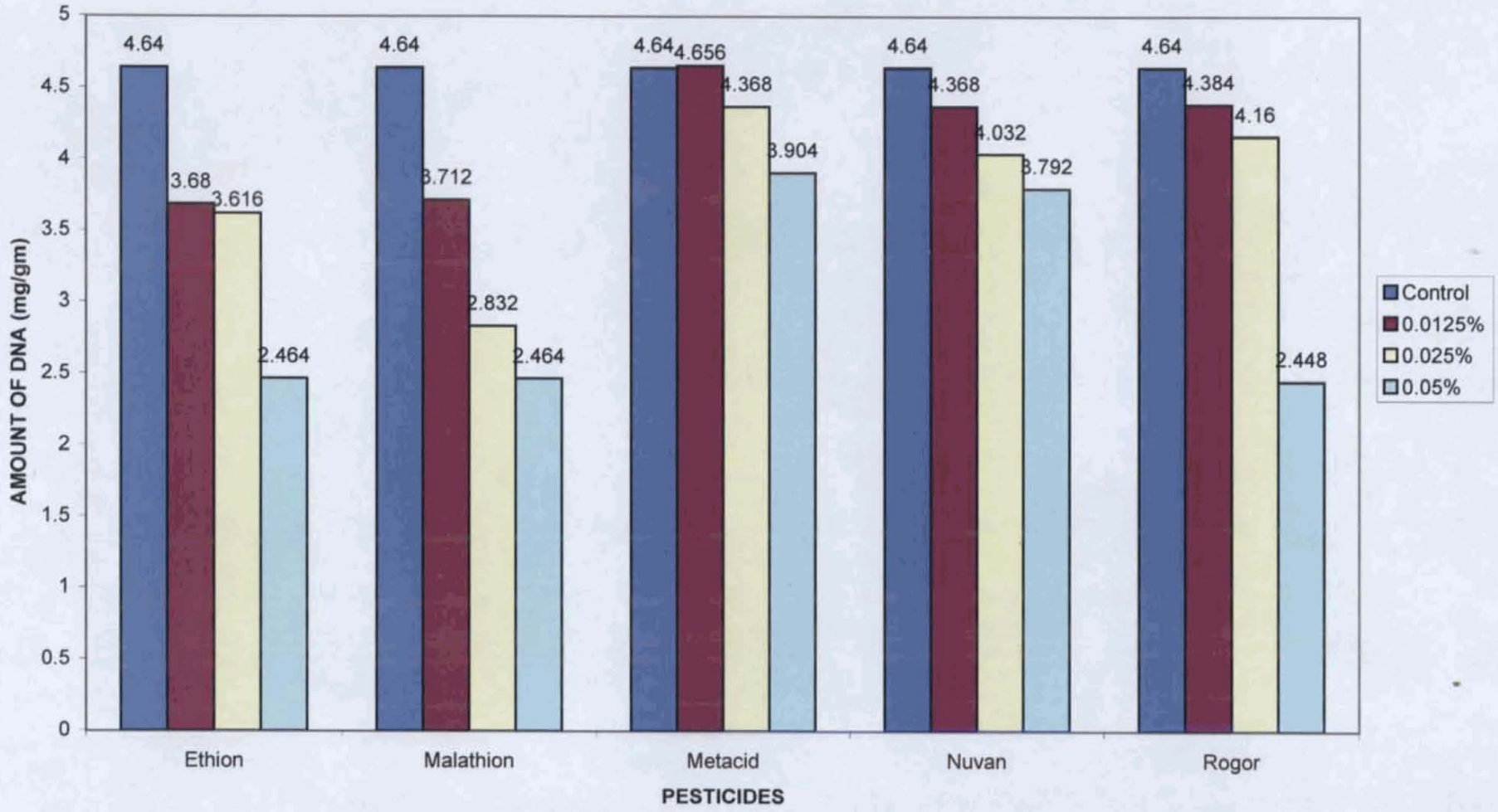
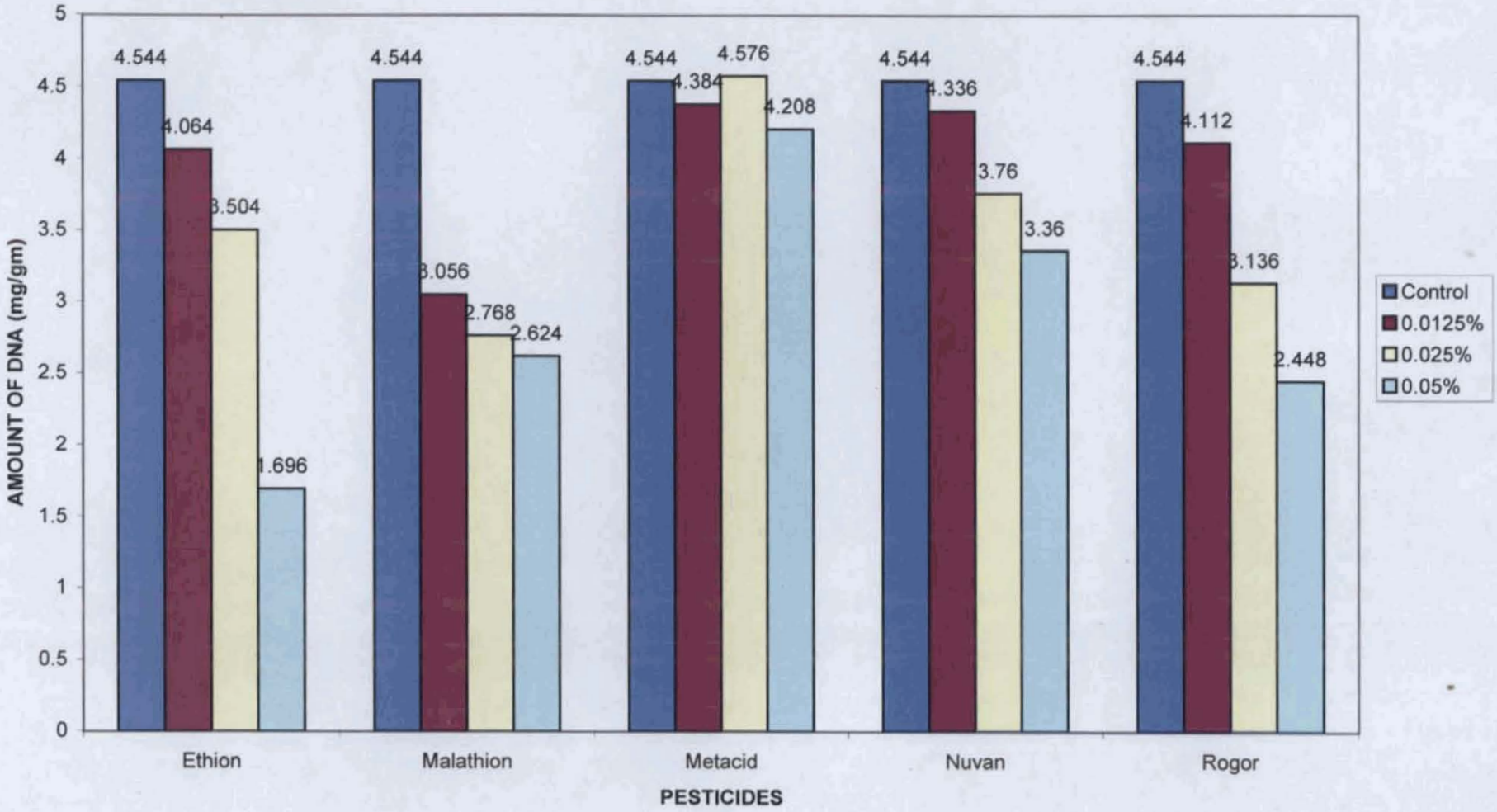


FIG. 126. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON THE AMOUNT OF DNA IN ROOT TIP CELLS OF *ALLIUM CEPA* AFTER 2 HOURS OF TREATMENT



Extraction of samples: -

Before estimation there should be extraction of proteins from samples. For this 100 mg of the fresh treated samples of each were ground well separately in a mortar and pestle with a small quantity of acid washed sand. 5 ml of 0.2 M. ice-cold phosphate buffer (pH 7.2) was added to the homogenate. The homogenate was centrifuged for 5 minutes at 5000 rpm in room temperature. 5 ml of 10% ice cold TCA was added to the homogenates and they were allowed to stand for 15 minutes on ice and then centrifuged again at 5000 rpm for 5 minutes. Supernatants were discarded and 2 ml of 2% TCA was added to the precipitates and stirred well and centrifuged at 5000 rpm for 5 minutes. After that the precipitates were washed with 5 ml of 80% acetone and centrifuged it and discarded the supernatants. 5 ml of 100 % acetone was added to the precipitates and centrifuged as usual. Supernatants were discarded and the precipitates exposed to air for 20 minutes for drying.

For the estimation of protein, 2 ml of 0.1 N NaOH was added to the precipitates and kept on a boiling water bath for 5 minutes. Five ml of alkaline solution was added to an aliquot taken from the above protein extracts. The mixtures were vigorously shaken well and allowed to stand for 10 minutes at room temperature. 0.5 ml of 1N Folin-Ciocalteau reagent was added to the above sample and mixed well. It was then incubated at room temperature in the dark for 30 minutes. Blue colour developed. Absorbance was measured at 750 nm by using Bausch and Lomb model spectrophotometer.

Preparation of standard graph: -

0.2, 0.4, 0.6, 0.8 and 1ml of the working standards were pipetted into a series of test tubes. Duplicates were also taken separately. The volumes were made up to 1 ml in all the test tubes with distilled water. A tube with 1ml of distilled water served as the blank. 5 ml of alkaline copper solution was added to

each tube including the blank. These were mixed well and allowed to stand for 10 minutes. 0.5 ml of Folin-Ciocalteau reagent was added and mixed well. All these tubes were incubated at room temperature in the dark for 30 minutes. Blue colour developed. The absorbances were measured at 750 nm. The standard graph was prepared and the amount of protein present in one gram of the samples was calculated by using the formula given below.

$$\frac{\text{Amount of standard protein}}{\text{Its optical density}} \times \text{OD of the particular treated sample} \times \frac{\text{Total volume of the sample}}{\text{Volume of aliquot}} \times \frac{1000}{100}$$

3. ESTIMATION OF TOTAL FREE AMINO ACIDS

It was done according to Lee and Takahazhi (1966).

Reagents required: -

- 1% Ninhydrin: - 1g of Ninhydrin was dissolved in 100 ml. of 0.5 molar citrate buffer at pH 5.5.
- Citrate buffer: - It was prepared by dissolving 1.4705 gms. of sodium citrate and 1.0505 gms. of citric acid in 100 ml. of distilled water, each in separate glass vessels. 15 ml. of citric acid was mixed with 35 ml. of sodium citrate and diluted to a total volume of 100 ml. and checked its pH. with a pH. meter and was found to be 5.5.
- Glycerol.
- Standard amino acid: - Glycine
- Ninhydrin citrate glycerol mixture: - It was prepared by mixing 1 ml. of 1% ninhydrin in citrate buffer, 2.4 ml. of glycerol, and 0.4 ml. citrate buffer at pH 5.5.

Extraction of amino acids from the sample: -

100 mg of the treated root tips were weighed accurately, ground in a mortar and pestle by adding a known volume of 80% alcohol and a small

quantity of acid washed sand. This homogenate was taken and centrifuged at 5000 rpm for 5 minutes in room temperature and the supernatants were saved. The extractions of samples were repeated twice with the residues and pooled all the supernatants in separate test tubes according to their treatment concentrations. The volumes of the extracts were reduced to 1 ml by evaporation and used for quantitative estimation.

Preparation of standard graph and estimation of total free amino acid: -

For the preparation of standard graph, a 20 mM. solution of amino acid glycine was prepared by dissolving 15.014 mg / 10 ml of distilled water as the standard stock, and working standards were prepared by diluting the standard with distilled water and prepared a 0.2 ml of solution carrying amino acids of 0.025 μ M, 0.05 μ M, 0.1 μ M and 0.2 μ M. 3.8 ml of ninhydrin-citrate-glycerol mixture was added to the above. The final pH of the reaction mixture was 6. This reaction mixture was heated in a boiling water bath for 12 minutes and cooled in a tap water bath at room temperature. The test tubes were shaken well and the absorbance taken at 570 nm on Bausch and Lomb model spectrophotometer within 1 hour and the standard graph was prepared.

For the estimation of amino acid present in the samples, 0.2 ml of each sample extracts were treated just like the standard and measured the absorbance at 570 nm within 1 hour. The reagent blank and the standard amino acid solutions were run at the same time to verify and standardize the determination. The amounts of total free amino acids were expressed as glycine equivalents gm^{-1} fresh weight.

RESULTS

Kochuthressia M. V. "Cytotoxic and biochemical effects of some organophosphorus pesticides in Allium cepa L. and Allium sativum L." Thesis. Department of Botany, University of Calicut, 2005

RESULTS

A. CYTOTOXIC EFFECTS

The effects of different treatments with the five organophosphorus pesticides such as Ethion, Malathion, Metacid, Nuvan and Rogor on mitotic divisions in the root tip meristems of *Allium cepa* and *Allium sativum* in two different time durations (1 and 2 hours) are given in tables 1-10. In the present investigation, *A. cepa* and *A. sativum* root tip cells showed normal mitotic division when treated with distilled water as control (Figs. 1-8). The mitotic indices showed a gradual decrease in one-hour and two-hours of treatment. But all the divisional stages observed were normal. A considerable extent of mitotic inhibition and a wide spectrum of chromosomal aberrations were observed on *A. cepa* and *A. sativum* root tip cells after treatment with the five organophosphorus pesticides. The mitotic indices decreased (Figs.117-120) and the abnormalities increased (Figs. 121-124) with the durations and concentrations of the treatment. The frequency of occurrence of abnormalities and the value of mitotic indices were found to be different in both the plant materials. These test materials showed many chromosomal abnormalities after each treatment. The major abnormalities observed in *Allium cepa* were nuclear lesions at interphase, elongated nuclei showing prominent lesions, sickle shaped nucleus, interphase cell with a micronucleus, interphase nucleus with many nuclear fragments, unequal nuclear fragments, binucleate cell, binucleate cell with two lesions in each nucleus, dumbbell shaped nucleus, multiple nuclei in a cell showing deformation, nuclear extrusions, misorientation of chromosomes at metaphase, highly irregular chromosomes, chromosome fragments and partial stickiness during metaphase, dislocation of chromosomes at metaphase, sticky metaphase, diagonal C-metaphase, diagonal metaphase showing chromosome stickiness, stickiness and dislocation of a chromosome at metaphase, star metaphase, C-metaphase, clumping of chromosome at metaphase, ball metaphase with a

prominent lesion, unequal segregation and abnormal association of chromosome during metaphase, chromosome erosion and scattering, chromosome pulverization, stickiness at anaphase, sticky anaphase showing multiple bridges, ring chromosomes, multipolarity and unequal segregation of chromosomes, sticky anaphase with bridges, stathmo anaphase, chromosome stickiness at anaphase with a bridge and a ring chromosome, stickiness and bridges, bridges and non-orientation, anaphase with dislocation of chromosomes, bridges and early movement at anaphase, diagonal anaphase with early movement, anaphase showing early movement, diagonal anaphase, anaphase with two chromosome bridges, diagonal anaphase with bridges, lagging chromosome fragments and a ring chromosome, multipolarity, early movement and a chromosome ring, diagonal sticky anaphase with a single chromosome bridge, star anaphase, anaphase erosion, stickiness and dissolution of chromosomes during anaphase, stickiness and laggard at telophase, aberrant telophase with a lagging chromosome, two parallel bridges at telophase, diagonal telophase, multiple bridges at telophase, non-orientation and stickiness, sticky telophase with two micronuclei, chromosome stickiness with double bridges at early telophase, telophase with sticky bridge, sticky telophase with a bridge and a ring chromosome and also a polyploid cell (Figs. 9-68). Whereas, in *Allium sativum*, the abnormalities noticed were abnormal prophase, nuclear lesion, interphase cell with micronucleus, binucleate cell, binucleate cell with nuclear lesion, binucleate cell with nuclear extrusion, nuclear deformation, ball metaphase, chromosome stickiness at metaphase, chromosome clumping, sticky irregular metaphase with chromosome fragments, diagonal metaphase, sticky metaphase showing chromosome erosion, sticky metaphase in a hyperploid cell, oblique metaphase, misorientation of chromosomes, C-metaphase, anaphase with lagging chromosomes, diagonal anaphase with early movement and a dislocated

chromosome, anaphase with two ring chromosomes, multiple bridges, obliquely placed star anaphase, diagonal anaphase with early movement, disturbed chromosomes, diagonal stathmo-anaphase, diagonal anaphase, anaphase with early movement and bridge formation, diagonal sticky anaphase, multipolarity with chromosome fragments at anaphase, multipolarity at anaphase, cell showing unequal segregation of chromosomes, tripolarity with persistent bridges at late anaphase, sticky star anaphase, unequal separation, misorientation and non-synchronous groups of chromosomes at anaphase, anaphase with bridges and early movement, stathmo-anaphase, chromosome bridges and dislocated chromosomes, bridge at late anaphase, non-synchronous movement and scattering of chromosomes at anaphase, sticky anaphase with chromosome fragment and bridges, tripolarity and chromosome laggard at anaphase, sticky bridge and a deserted chromosome, tripolarity and unequal groups of chromosomes at telophase, sticky telophase, diagonal sticky telophase and also chromosome bridge and laggard at telophase (Figs. 69-116). The most frequent abnormalities observed were stickiness, clumping, C-metaphase, early movement, bi-nucleate cells, diagonal orientation of chromosomes at metaphase, anaphase and telophase, bridges at anaphase and telophase and also nuclear lesions at interphase. Few mitotic abnormalities were found to be common in both the test materials.

The mitotic indices in the various treatments on both the plant materials were found to be less than that of the controls. Normally the frequencies of abnormalities were found to increase with the increasing concentrations of the pesticides and with the time duration. But certain exceptions were also observed. The percentage of cytotoxicity and mitotic inhibition were found to be different in *A. cepa* and *A. sativum*. The former shows more cytotoxicity and inhibition than the latter. The mitotic indices ranged from 21.29 to 9.76 in *A. cepa* (Figs.

117-118) and from 19.37 to 9.55 in *A. sativum* (Figs.119-120). The mitotic abnormalities ranged from 0.68% to 2.7% in *A.cepa* (Figs. 121-122) and from 0.54% to 2.39% in *A. sativum* (Figs.123-124).

1. The treatment of *Allium cepa* root tip cells with Ethion: -

The mitotic indices on the root tip cells of *A. cepa* were found to be decreased with the increase in concentration and time duration. Mitotic indices were found to be higher in one-hour treatments (21.45-12.69; Fig. 117) than the 2-hour treatments (20.13-12.10; Fig. 118). Control showed no abnormal cells. 0.0125% of Ethion exhibited diagonal and sticky metaphase, fragmentation and partial stickiness at metaphase, stickiness and dislocation, clumping, irregular metaphase, early movement of chromosomes at anaphase, diagonal anaphase with bridges, stickiness and bridges, stickiness at telophase and aberrant chromosome with lagging. One-hour treatment of 0.0125% Ethion produced 1.14% of abnormal cells (Fig 121); where as 2-hour treatment in the same concentration produced 1.31% abnormality (Fig.122).

0.025% of Ethion showed stickiness and dislocation, clumping, early movement at anaphase, diagonal anaphase, stickiness with bridges, stickiness and laggards at telophase, multiple bridges, nuclear extrusion, micronuclei and binucleate cell. The percentage of abnormal cells was 1.33 at one-hour treatment and 2.26 at two-hours treatment. 0.05% of Ethion produced 1.41% and 2.36% of abnormal cells in one and two-hours of treatments respectively. The abnormalities produced by the above concentration were diagonal and sticky metaphase, fragmentation and partial stickiness, stickiness and dislocation at metaphase, clumping, ball metaphase with a lesion, diagonal anaphase, diagonal anaphase with bridges, stickiness and bridges at anaphase, multiple bridges, nuclear extrusion, micronuclei and binucleate cells.

Treatment of *A. cepa* root meristems in 0.1% of Ethion induced 1.56% and 2.7% of abnormal cells in one and two-hours treatments respectively. It also showed various chromosomal aberrations such as diagonal and sticky metaphase, stickiness and dislocation, irregular metaphase, diagonal anaphase, diagonal anaphase with bridge, stickiness with bridge, stickiness at telophase, telophase with aberrant chromosome and multiple bridges, micronuclei and binucleate cells.

Mitotic index and frequency of abnormal cells showed gradual decrease and increase respectively as the concentration of Ethion increased (Table-1). One-hour treatment showed comparatively lesser abnormalities than the 2 hours treatment (Figs. 121-122). Metaphase stickiness and dislocation, clumping, sticky anaphase with bridges and stickiness at telophase were the major abnormalities noticed when root tip cells were treated with 0.0125%, 0.025%, 0.05% and 0.1% of Ethion.

2.Treatment of *Allium sativum* root tip cells with Ethion: -

The cytotoxic activities of Ethion on *A. sativum* were analysed. Different treatments of Ethion *on A. sativum* root tip meristem showed various chromosomal aberrations such as diagonal metaphase, sticky irregular fragments, stickiness at metaphase, C-metaphase, misorientation, clumping of chromosomes, diagonal anaphase with early movement, multipolarity, bridges and early movement, lagging chromosome fragments, stickiness at telophase, diagonal and sticky telophase, tripolarity and unequal groups of chromosomes, bridge and laggard, nuclear lesions, and bi-nucleate cells. The mitotic index of 1-hour treated cells ranges from 14.87 to 10.39 (Fig. 119). Whereas the 2-hour treated materials showed mitotic index which range from 15.12 to 10.66 (Fig.120; Table-2). Percentages of abnormal cells were directly proportional to the concentrations of the pesticide used. It ranged from 0.69% to 1.63% in one-

hour treatment (Fig.123) and 0.77% to 1.85%in two-hours treatment (Fig.124). Diagonal metaphase and diagonal anaphase with early movement were observed in all the concentrations of pesticide during all the treatment durations. Sticky irregular fragments, C-metaphase, metaphase clumping, tripolarity and unequal groups of chromosomes were present in the lower concentrations of Ethion. Stickiness was more frequent in the higher concentrations. Bridge and laggards at telophase were noticed in the concentration of 0.0125%, 0.025% and 0.1%. The percentages of abnormalities were found to be directly proportional to the increase in concentrations of Ethion where as the mitotic index exhibits an inverse relationship with the increase in concentration of Ehion on *Allium sativum*.

The root tip cells of pesticide treated samples revealed several cyto and chromo toxic effects, which are presented in Table 2. Bridges and early movements were observed to the maximum in 0.05% of Ethion treatment at two-hours. Nuclear lesions and binucleate cells were more predominant in the higher concentrations of Ethion. The percentages of abnormal cells in *A. sativum* after the treatment with Ethion were comparatively less than that of *A. cepa*, especially after prolonged treatment.

3.Treatment of *Allium cepa* root tip cells with Malathion: -

The results obtained from the squash preparations of root tips exposed to various insecticide concentrations of Malathion for one and two-hours are summarised in Table-3. The mitotic index decreased with the increase in insecticide concentration applied. It ranged from 18.43 to 12.55 in one-hour treatment (Fig.117) and from 15.88 to 11.69 in two-hours treatment (Fig.118). Chromosomal aberrations like stickiness and dislocation, unequal segregation and abnormal association, diagonal metaphase and stickiness, C-metaphase, sticky anaphase and multiple bridge, chromosome ring, diagonal anaphase and

early movement, anaphase with double bridges, multipolarity, early movement and ring, stickiness, dislocation of chromosomes, diagonal anaphase with bridge, multiple bridges at telophase, elongated nucleus with lesions and bi-nucleate cells with nuclear lesions were found. Cytotoxicity was found to be increasing with the increase in the insecticide concentration. Percentage of abnormality ranges from 0.88 to 1.28 in one-hour treatment (Fig.121) and from 1.22 to 2.09 in two-hours treatment (Fig.122). Stickiness and dislocation, unequal segregation and abnormal association and chromosome ring in anaphase comprised the most dominant type of the anomalies observed in the root meristems of *A. cepa* after treatment with Malathion in different concentrations. C-metaphase was found in all the treatments during 1 hour. In two-hours of treatment, it was present in 0.025% and 0.05% of Malathion treated cells of *A. cepa*. Elongated nucleus with lesions and binucleate cells with nuclear lesions were also observed in the interphase cells after treatment with the different concentrations of the pesticide. Malathion treatments induced cells with chromosome abnormalities, which increased in number as the concentration of the insecticide increased (Table-3). The used concentrations of Malathion caused decrease in the mitotic index of *A. cepa* root meristems when compared with the control.

4. Treatment of *Allium sativum* root tip cells with Malathion: -

The different concentrations of Malathion caused decrease in the mitotic index of *Allium sativum* root meristems. The mitotic index ranges from 16.8 to 11.84 in one-hour treated cells of *A. sativum* (Fig.119) and from 14.09 to 12.38 after two-hours treatment (Fig. 120). Malathion treatments induced cells with chromosome abnormalities, which increased in number as the concentration of the insecticide increased (Table-4). The abnormal cells range from 1.08% to 1.51% in one-hour treatment (Fig.123) and from 0.98% to 1.71% in two-hours treatment (Fig.124). Treatments with the different concentrations of Malathion

FIG. 127. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON THE AMOUNT OF DNA IN ROOT TIP CELLS OF *ALLIUM SATIVUM* AFTER 1 HOUR TREATMENT

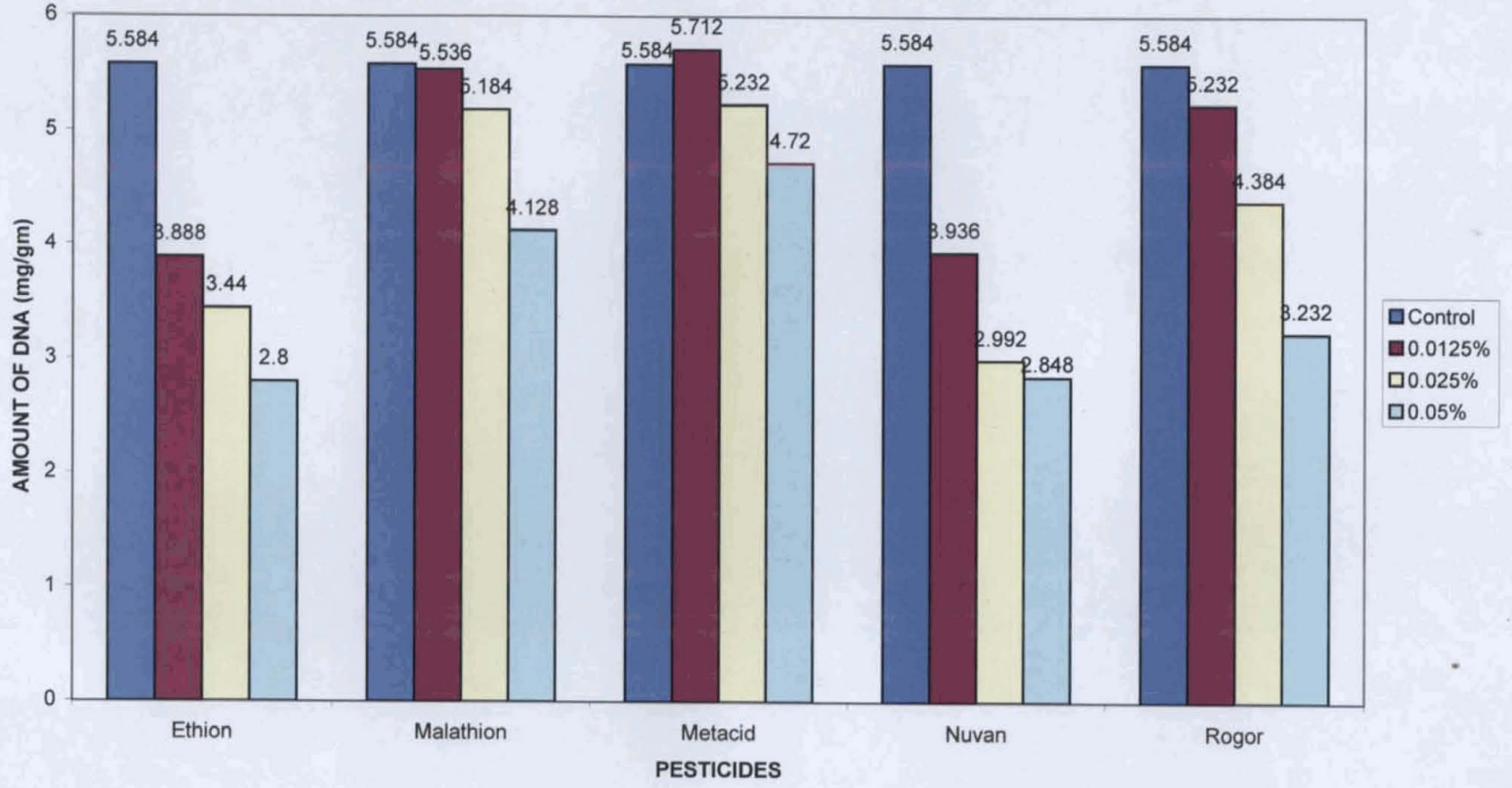


FIG. 128. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON THE AMOUNT OF DNA IN ROOT TIP CELLS OF *ALLIUM SATIVUM* AFTER 2 HOURS OF TREATMENT

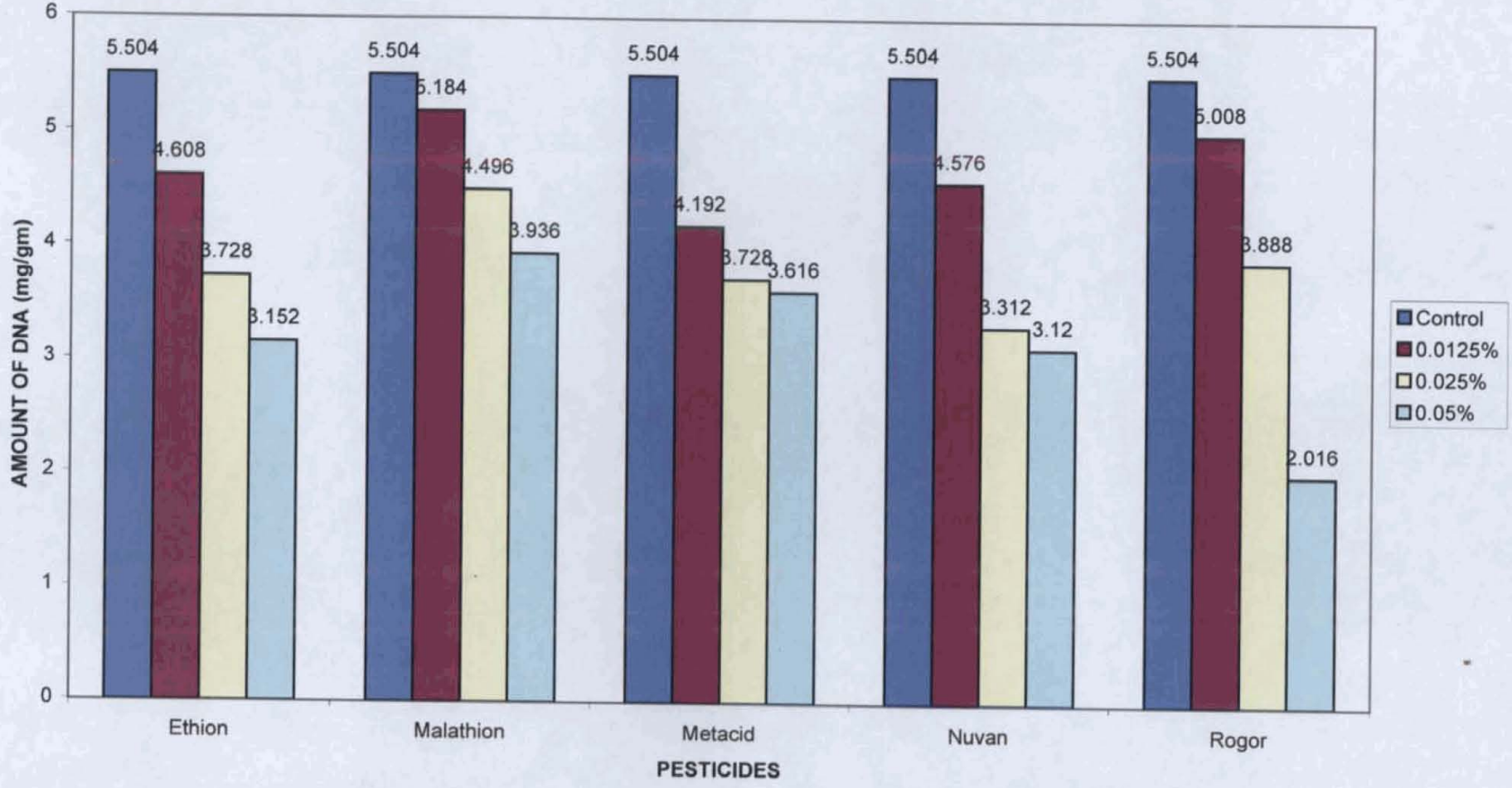


FIG. 129. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON THE AMOUNT OF RNA IN ROOT TIP CELLS OF *ALLIUM CEPA* AFTER 1 HOUR TREATMENT

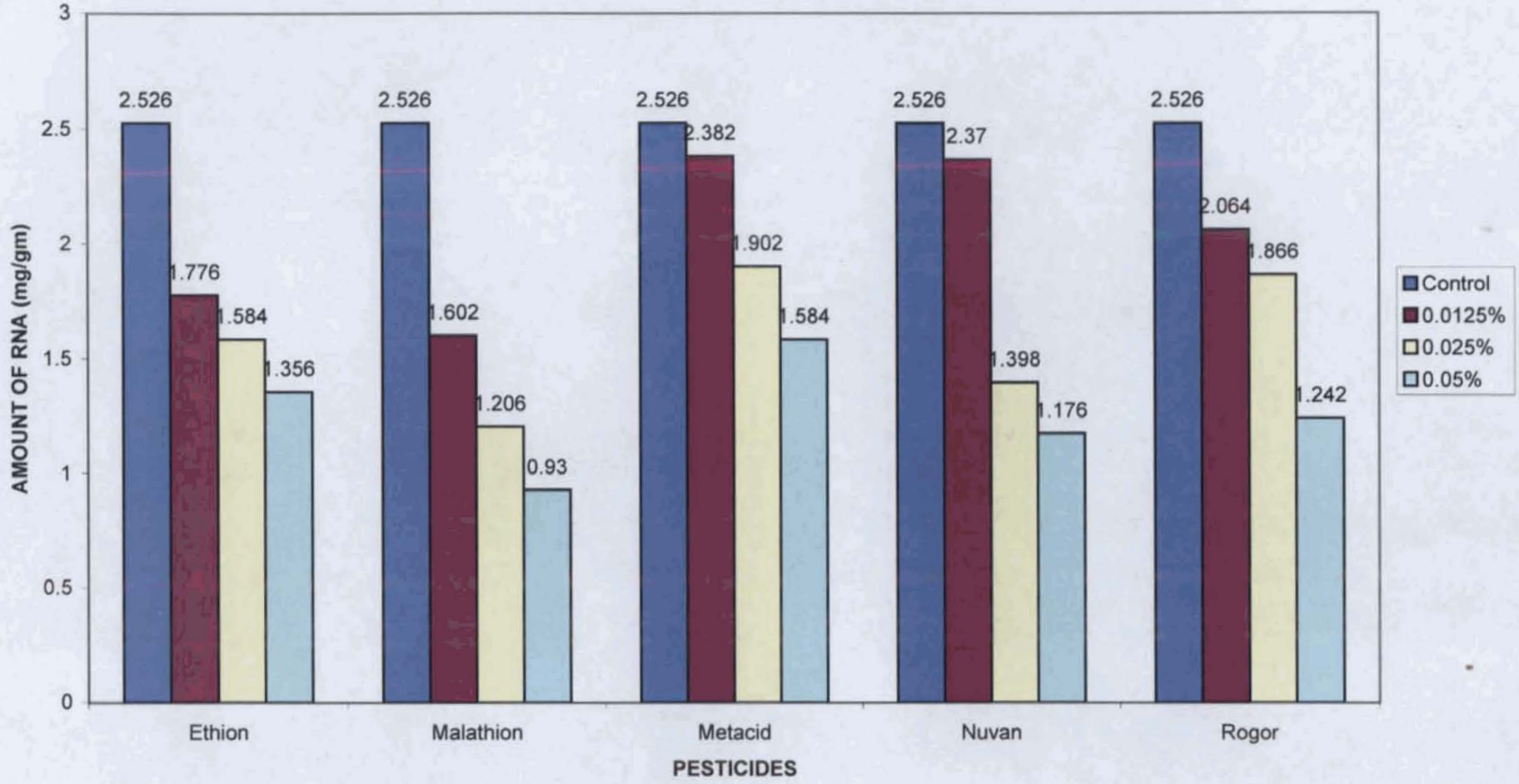
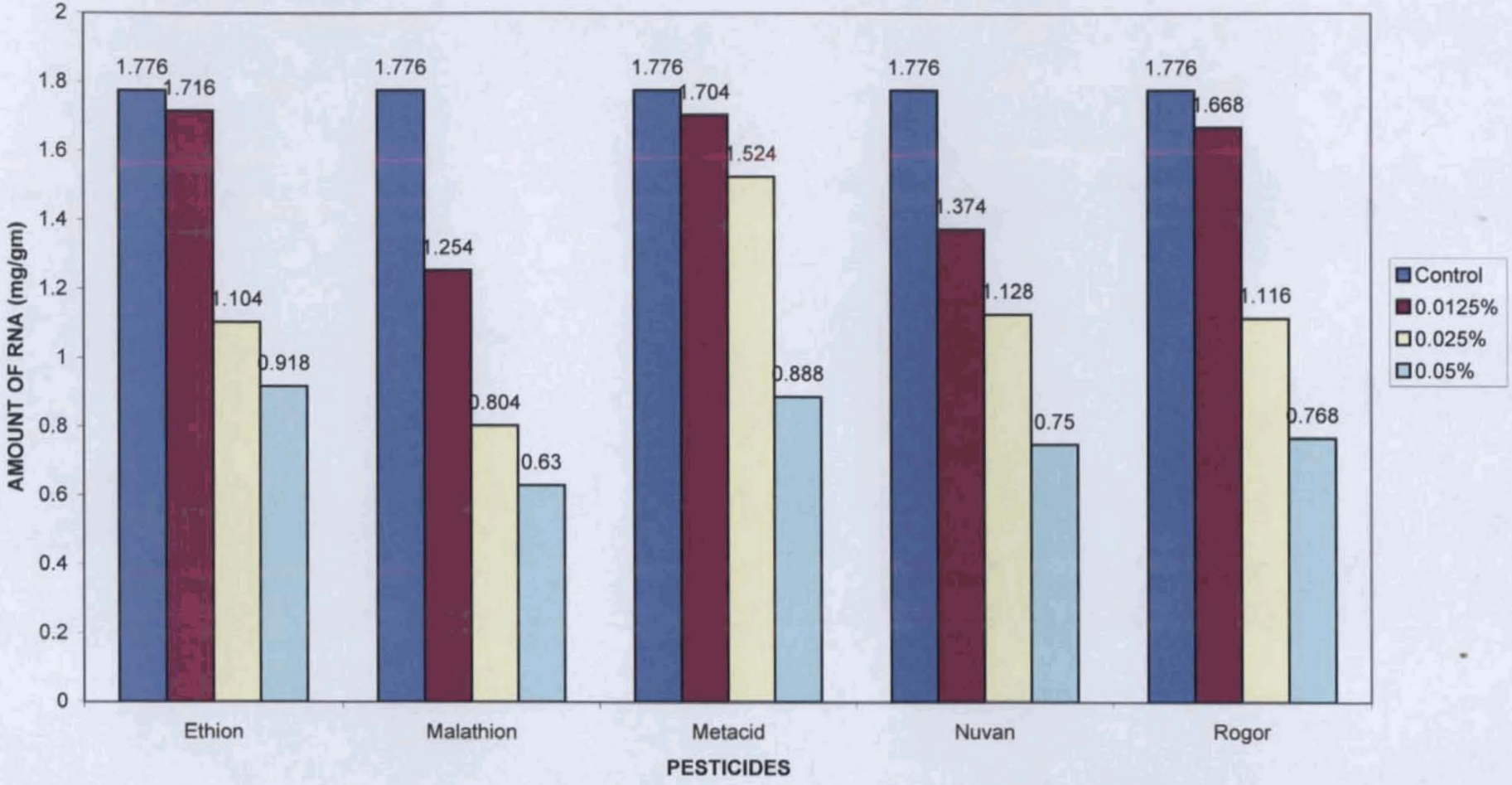


FIG. 130. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON THE AMOUNT OF RNA IN ROOT TIP CELLS OF *ALLIUM CEPA* AFTER 2 HOURS OF TREATMENT



induced abnormal prophase, diagonal metaphase, oblique metaphase, stickiness at metaphase, ball metaphase, clumping, stickiness showing erosion, tripolarity with bridges, late anaphase bridges, multipolarity, ring chromosomes, stickiness at telophase, diagonal and sticky telophase, tripolarity and laggard, bridge at telophase, micronucleus and binucleated interphase cells. Interphase cells with nuclear deformations were also observed after treatment with the different concentrations of the insecticide. Abnormal prophase was found only in the treatment of 0.0125% of pesticide during 1 hour. Diagonal metaphase, clumping of chromosomes and stickiness and sticky diagonal telophase were present in almost all the treatments with different concentrations of the pesticide Malathion. The ring chromosomes were found only in the concentration of 0.025% of one-hour and 0.05% of 2-hour treatment. Diagonal and sticky telophase was noticed in almost all the concentrations except 0.0125% of Malathion during two-hours treatment. Interphase abnormalities such as micronucleus, bi-nucleate cells and nuclear deformations were observed after the treatment with the different concentration of insecticide. The frequencies of aberrations exhibit a linear increase with the increase in duration of the treatments. In all the treatments, the decrease in mitotic index values in the root meristems of *A. sativum* with increasing concentrations of Malathion was attributed to mitotic inhibition.

5. Treatment of *Allium cepa* root tips with Metacid: -

Metacid treatments reduced the mitotic index in *A. cepa* as the concentration of the pesticide increases (Table-5). The effect of Metacid was more pronounced that it interferes with the normal sequence of cell cycle to reduce the number of cells starting to divide at interphase. Various concentrations ranging from 0.0125% to 0.1% were used in the experiments but higher concentrations of Metacid appeared to be causing drastic changes within two-hours treatment in *A. cepa.* Like Malathion, Metacid also decreased the

division rate in all the concentrations tried and the reduction appeared to be dose dependant. The mitotic index ranged from 17.95 to 11.19 in one-hour treatment (Fig.117) and from 17.93 to 11.17 in two-hours treatment (Fig. 118). Pesticide induced chromosomal abnormalities such as stickiness of chromosomes at metaphase, fragmentation and partial stickiness, diagonal C-metaphase, star metaphase, metaphase stickiness and dislocation, polyploidy, lagging fragments and ring, anaphase stickiness and triple bridges, sticky diagonal bridges, early movement, dissolution and stickiness of chromosomes, stickiness with two micronuclei at telophase, stickiness and double bridges, sickle shaped nucleus, unequal nuclear fragments and nuclear lesions. Out of these chromosomal aberrations, metaphase stickiness and polyploidy were frequent and are found in almost all the treatment concentrations. Diagonal C-metaphases were noticed in all the treatments except in 0.025% of Metacid for one-hour. Star metaphases were also found in all the treatment concentrations except in 0.05% of the pesticide for two-hours. Stickiness and dislocation were observed in all the experiments except in 0.0125% Metacid for two-hours. Interphase showed sickle shaped nucleus, unequal nuclear fragments and nuclear lesions in low frequencies. The root treatments with different concentrations of Metacid induced a significant percentage of abnormal mitosis. The percentage of abnormalities increased as the concentration of the pesticide increases. It ranges from 0.99% to 1.97% in One-hour treatment (Fig.121) and from 1.24% to 2.08% in two-hours treatment (Fig.122).

6.Treatment of *Allium sativum* root tip cells with Metacid: -

The results observed from root tips of *A. sativum* exposed to various insecticide concentrations for one-hour and two-hours are summarised in the Table-6. The mitotic index decreased with the increase in insecticide concentrations applied. It ranges from 14.05 to 11.61 in one-hour treatment

(Fig.119) and from 13.27 to 9.82 in two-hours treatment (Fig.120). Cytotoxicity was found to be increasing with the increase in the insecticide concentration. The percentage of abnormality ranges from 0.58% to 1.73% in one-hour treatment (Fig.123) and from 0.88% to 2.39 % in two-hours treatment (Fig.124). The abnormalities include diagonal metaphase, stickiness in hyperploid cells, stickiness at metaphase, misorientation, clumping, diagonal early movement and dislocation at anaphase, diagonal stathmo-anaphase, ring chromosomes, bridges and dislocation, oblique star anaphase, disturbed chromosomes at anaphase, diagonal and early movement, multiple bridges, anaphase stickiness with bridge and fragment, stickiness at telophase, diagonal and sticky telophase, bridges at telophase and also tripolarity and unequal groups of chromosomes. Micronuclei and bi-nucleate cells with nuclear extrusions were found to be present in the interphase cells. Chromosomal aberrations like stickiness at metaphase, diagonal stathmo-anaphases were found to be present in all concentrations. Clumping was recorded in almost all the treatment concentrations of pesticide in two different time durations. Cytotoxic aberrations were found to be more predominant during metaphase when compared with other stages of the cell cycle in *A. sativum* after treating with different concentrations of Metacid. The effects were always dose dependent and there exist a positive correlation between cytotoxicity and concentration of pesticides.

7. Treatment of *Allium cepa* root tip cells with Nuvan: -

The cytological examination of the root tip cells after the treatment with different concentrations of pesticide Nuvan revealed a wide spectrum of chromosomal aberrations. These aberrations were shown in Table 7. The abnormality was found to range from 0.96% to 1.82% in one-hour treatment (Fig.121) and from 0.92% to 2.01% in two-hours treatment (Fig.122). In the various treatments, metaphase stickiness and dislocation of chromosomes were

noticed frequently. Clumping of chromosomes, ball metaphase with lesion, irregular metaphase, C-metaphase, misorientation of chromosomes at metaphase, bridges and misorientation, anaphase erosion, dislocation, sticky bridges and ring, misorientation and stickiness during telophase, diagonal telophase, parallel bridges, stickiness and micronuclei, and bi-nucleate cells were noticed in low frequencies and their presence or absence in the different treatments are also recorded in Table-7. Stickiness and dislocation becomes more frequent in all concentrations of pesticide studied. The mitotic index ranges from 18.79 to 12.28 in one-hour treatment (Fig.117) and from 14.96 to 10.26 in two-hours treatment (Fig.118). The mitotic index and the percentage of abnormalities were negatively correlated with each other.

8. Treatment of *Allium sativum* root tip cells with Nuvan: -

The most common chromosomal aberration observed after treating Nuvan on *A. sativum* root tip cells was stickiness at metaphase. Summary of treatments are presented in Table 8. Abnormal cells range from 0.54% to 1.35% in one-hour treatment (Fig.123) and from 0.7% to 1.51% at two-hours treatment. Considering the different mitotic stages, it could be observed that, there were increases in the percentage of abnormal cells when the concentration of Nuvan increases.

Nuvan induces a number of abnormalities involving all the stages of mitosis except prophase. Chromosome stickiness at metaphase and telophase was the most common type of abnormalities that appeared in all the treatments. Other types of abnormalities were sticky irregular fragments, diagonal metaphase, C-metaphase, bridges and early movement at anaphase, multipolarity, early movement, diagonal and sticky anaphase, unequal separation, misorientation and non-synchronous movement of chromosomes, stathmo-anaphase, sticky star anaphase, diagonal and sticky telophase, bridge at telophase and nuclear lesion at interphase. The pesticide Nuvan has the capacity to arrest cell division process at

mitosis. Because of this, mitotic indices decrease as concentration and duration of pesticide treatment increase. The mitotic indices of Nuvan treated cells decreased from 14.26 to 9.81 during one-hour treatment (Fig.119) and from 13.78 to 10.48 during two-hours treatment (Fig.120).

9.Treatment of *Allium cepa* root tip cells with Rogor: -

The root tip cells of *A. cepa* after treatment with Rogor showed abnormal mitosis with various types of abnormalities. Among the different abnormalities observed, anaphase abnormalities were more frequent. Mitotic index decrease as concentration and duration of pesticide treatment increase. It decreased from 15.34 to 11.88 in one-hour treatment (Fig.117) and from 13.41 to 9.76 in two-hours treatment (Fig.118). Rogor was found to induce mitodepression, spindle abnormalities and structural aberrations of chromosomes even in lower concentration. The relative percentage of abnormality ranges from 0.83% to 2.07% in one-hour treatment (Fig.121) and from 0.68% to 2.11% in two-hours treatment (Fig.122). Abnormalities like chromosome stickiness, dislocation, erosion and scattering of chromosome, pulverization, star anaphase, double bridge, diagonal anaphase, bridge and early movement, multipolarity and unequal segregation, lagging fragments and ring chromosome, stathmo anaphase, stickiness with two micronuclei at telophase, misorientation and stickiness, stickiness with bridge, multiple nuclei and nuclear deformation, dumbbell shaped nuclei and unequal nuclear fragments were observed. Double bridges and diagonal anaphase were recorded in the anaphase stage of *A. cepa* in all the concentrations except 0.0125% of Rogor at two-hours. Bridges and early movement of chromosomes were found in all the concentrations except 0.1% at one-hour and 0.0125% at 2-hour. Stickiness with two micronuclei at telophase was found in all the treatments except the concentration of 0.0125% at 2-hour treatment. Metaphase stickiness and stickiness with bridges at telophase were

present in all the treatments irrespective of duration. Multiple nuclei, nuclear deformation, dumbbell shaped nuclei and unequal nuclear fragments were very less frequent and they were found in the interphase cells.

The cytological observation from treated root tip cells revealed that Rogor had strong mitodepressive effect on *Allium cepa* root tip meristem. A decrease in mitotic activity was clearly observed at high concentrations (Table-9). The degree of cytological aberrations in mitosis is regarded as one of the dependable criteria for estimating the effect of pesticide.

10.Treatment of *Allium sativum* root tip cells with Rogor: -

The effects of the various treatments of Rogor on *A. sativum* root tips were shown in Table-10. The treatments had a marked reducing effect on mitotic index values particularly at the higher concentrations. Prolonged treatments resulted in further inhibition of the mitotic index values. Mitotic indices decreased to a value of 10.37 after one-hour treatment with the highest value of 19.37 (Fig.119). These values range from 14.22 to 9.55 in two-hours treatment (Fig.120). It could be seen from the Table-10 that Rogor induced a wide range of mitotic irregularities. Their frequencies increased as the concentration increased and the duration of treatment increased.

The percentage of mitotic abnormalities ranged from 0.84% to 1.34% after one-hour treatment (Fig.123) and from 1.02% to 1.75% after two-hour treatment (Fig.124). The abnormalities observed were diagonal metaphase, stickiness at metaphase, clumping, unequal segregation, diagonal anaphase, stathmo-anaphase, bridges and early movement, diagonal and early movement, sticky bridge and deserted chromosome, non-synchronous movement and scattering of chromosomes, tripolarity and laggard, diagonal and sticky telophase, stickiness at telophase, laggard at telophase, bi-nucleate cells with lesions and micronuclei. Stickiness, clumping and diagonal metaphases were

present in all the treatments at one-hour and 2-hour. Diagonal anaphase and sticky telophases were noticed in all the treatments in both time durations. Bi-nucleate cells with lesions were found in most of the treatments. Micronuclei were recorded more in the higher concentrations at 2-hour treatment.

B. BIOCHEMICAL EFFECTS

Biochemical effects of the five organophosphorus pesticides such as Ethion, Malathion, Metacid, Nuvan and Rogor (0.0125%, 0.025 %, 0.05%), each in two time durations such as one and two-hours treatment on *Allium cepa* and *Allium sativum* were analyzed. As the period of treatment prolonged, higher concentrations were found to be lethal and thus 0.1% treatment was eliminated. The inhibition of cell division by these chemicals leads to a depressive action on DNA, RNA, buffer soluble protein and total free amino acids in the cells. Increased concentration and prolonged period of treatment resulted in the increased reduction in the amount of nucleic acids, buffer soluble protein and total free amino acids. The amounts of each were calculated with the assistance of standard graph prepared prior to the experiments. In general, the reduction in mitotic activity and reduction in the amount of DNA, RNA, buffer soluble protein, and total free amino acids in the root tips of *A. cepa* was more pronounced than *A. sativum*. The biochemical effects of these organophosphorus pesticides on root tips of *A. cepa* and *A. sativum* were depicted in tables 11–50 and are also represented in figures 125-140. Detailed results of estimation of DNA, RNA, buffer soluble protein and total free amino acids were recorded separately.

I. EFFECT ON DNA

1. Estimation of DNA after the treatment of *Allium cepa* root tip cells with different concentrations of Ethion: -

The effect of different concentration of Ethion in two time durations caused a clear decrease in DNA content in *A. cepa*. The reduction in the DNA level was pronounced as the concentration increased. Hence the concentrations such as 0.0125%, 0.025%, and 0.05% were selected. In *A. cepa*, the control material of one-hour treatment, exhibits 4.640 ± 0.016 mg/g fresh weight of DNA and in the two-hours of treatment, the control carries 4.544 ± 0.064 mg/g fresh weight of DNA. When the root tips are subjected to 0.0125%, 0.025% and 0.05% of Ethion in one-hour, the DNA content lowers to 3.680 ± 0.016 mg/g, 3.616 ± 0.016 mg/g and 2.464 ± 0.016 mg/g fresh weight respectively (Fig.125). The reduction in the DNA content continued as the concentration increased. This nature was also seen in the two-hours of treatment. Control was found to be with maximum DNA content and when the root tips were treated with different percentages of Ethion such as 0.0125%, 0.025% and 0.05%, the DNA content decreased to 4.064 ± 0.016 mg/g, 3.504 ± 0.016 mg/g and 1.696 ± 0.016 mg/g fresh weight respectively (Fig.126). Data observed has been summarized in table 11.There was a marked decrease of DNA content in both time durations.

2. Estimation of DNA after the treatment of *Allium sativum* root tip cells with different concentrations of Ethion: -

A. sativum root tip cells were treated with different concentrations of Ethion and their DNA content was estimated after one and two-hours. There was a decrease in the DNA content with increase in concentrations of the pesticide and there was a maximum decrease at 0.05% in both time durations. When the DNA content of the *A.cepa* with *A. sativum* was compared, the *A. sativum* was found to possess more DNA content than *A. cepa*. The total DNA yield was

evaluated after each treatment of Ethion such as 0.0125%, 0.025%, 0.05% and found that it was about 3.888 ± 0.032mg/g, 3.440 ± 0.032mg/g and 2.800 ± 0.064mg/g fresh weight respectively for one-hour treatment (Fig.127) and during the two-hours treatment with the same concentrations of pesticide it was about 4.608 ± 0.016mg/g, 3.728 ± 0.032mg/g and 3.152 ± 0.032 mg/g fresh weight (Fig.128). Ethion caused a decrease of DNA content at a relatively high dose level. The estimated DNA yields are shown in the table 12.

3. Estimation of DNA after the treatment of *Allium cepa* root tip cells with different concentrations of Malathion: -

DNA content was estimated after the treatment of *A. cepa* root tip cells with different concentrations of Malathion in two time durations and found that there was a decrease in DNA content with increase in concentrations. Concentrations of pesticide and DNA content were inversely proportional to each other. Controls of root tips in both time durations possessed maximum DNA content and when root tips were subjected to cytotoxic treatment with each of the pesticide concentration, there was a gradual decrease in the DNA content. The amount of DNA present in the treated materials range from 3.712 ± 0.016 mg/g to 2.464 ± 0.016 mg/g fresh weight for one-hour treatment (Fig.125) and from 3.056 ± 0.016 mg/g to 2.624 ± 0.016 mg/g for the materials treated for two-hours (Fig.126). The results are recorded in table 13.

4. Estimation of DNA after the treatment of *Allium sativum* root tip cells with different concentrations of Malathion: -

The amount of DNA was estimated after the treatments of *A. sativum* root tip cells with different concentrations of Malathion in two different time durations. The DNA content showed gradual decrease as the concentration of the pesticide increased. The materials treated with 0.0125%, 0.025%, 0.05% of the pesticide recorded the DNA content of 5.536 ± 0.032mg/g, 5.184 ± 0.032mg/g

and 4.128 ± 0.016 mg/g respectively at one-hour treatment (Fig.127), where as it was found to be 5.184 ± 0.016mg/g, 4.496 ± 0.032mg/g and 3.936 ± 0.032mg/g fresh weight at two-hours treatment (Fig.128). Malathion also recorded reduction in the amount of DNA content as the concentrations of the pesticide increased. Details of results are tabulated in table 14.

5. Estimation of DNA after the treatment of *Allium cepa* root tip cells with different concentrations of Metacid: -

Table 15 shows the amount of DNA estimated after the treatment with different concentrations of pesticide Metacid on *A. cepa* root tip cells in two different time durations. When the root tips were treated with lowest concentration of pesticide, the amount of DNA slightly increased than the control. The amount of DNA ranges from 4.656 ± 0.016mg/g to 3.904 ± 0.064mg/g fresh weights for one-hour treatment (Fig.125). Where as, in two-hours treatment, the amount of DNA range from 4.576 ± 0.048 mg/g to 4.208 ± 0.032 mg/g fresh weight respectively (Fig.126).

6. Estimation of DNA after the treatment of *Allium sativum* root tip cells with different concentrations of Metacid: -

The effects of the used pesticide on DNA content of *A. sativum* are given in table 16. There was a slight increase of DNA content at 0.0125% of treatment. The DNA content ranges from 5.712 ± 0.016mg/g to 4.72 ± 0.032mg/g at one-hour treatment (Fig.127). In the two-hours treatment, the amount of DNA ranges from 4.192 ± 0.048 mg/g to 3.616 ± 0.048 mg/g (Fig.128).

7. Estimation of DNA after the treatment of *Allium cepa* root tip cells with different concentrations of Nuvan: -

The DNA quantified spectrophotometrically from the root tip cells of pesticide treated samples of *A. cepa* in one-hour and 2 hours treatment revealed a clear dose dependent decrease in the DNA content. The treated materials

FIG. 131. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON THE AMOUNT OF RNA IN ROOT TIP CELLS OF *ALLIUM SATIVUM* AFTER 1 HOUR TREATMENT

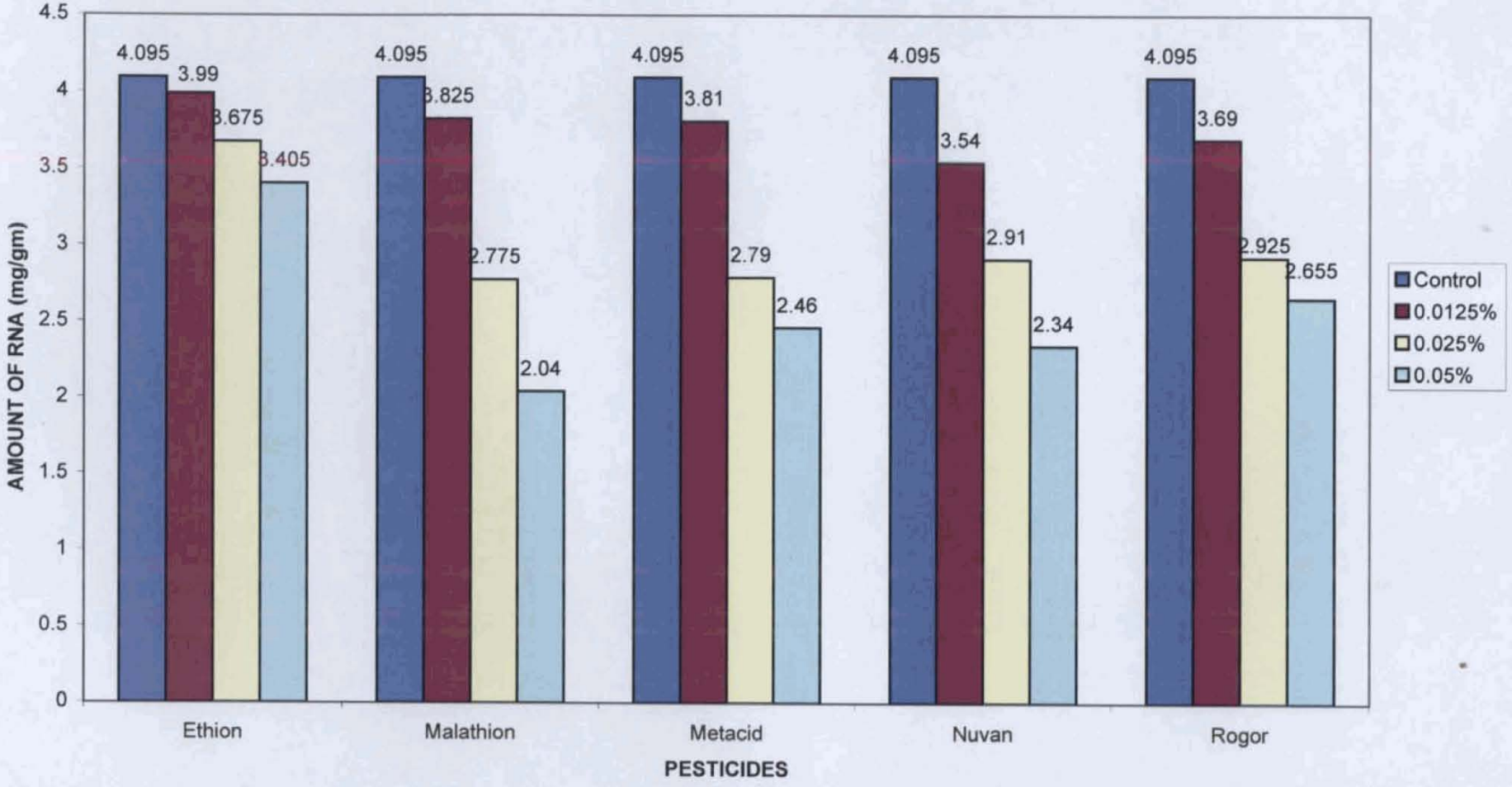


FIG. 132. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON THE AMOUNT OF RNA IN ROOT TIP CELLS OF *ALLIUM SATIVUM* AFTER 2 HOURS OF TREATMENT

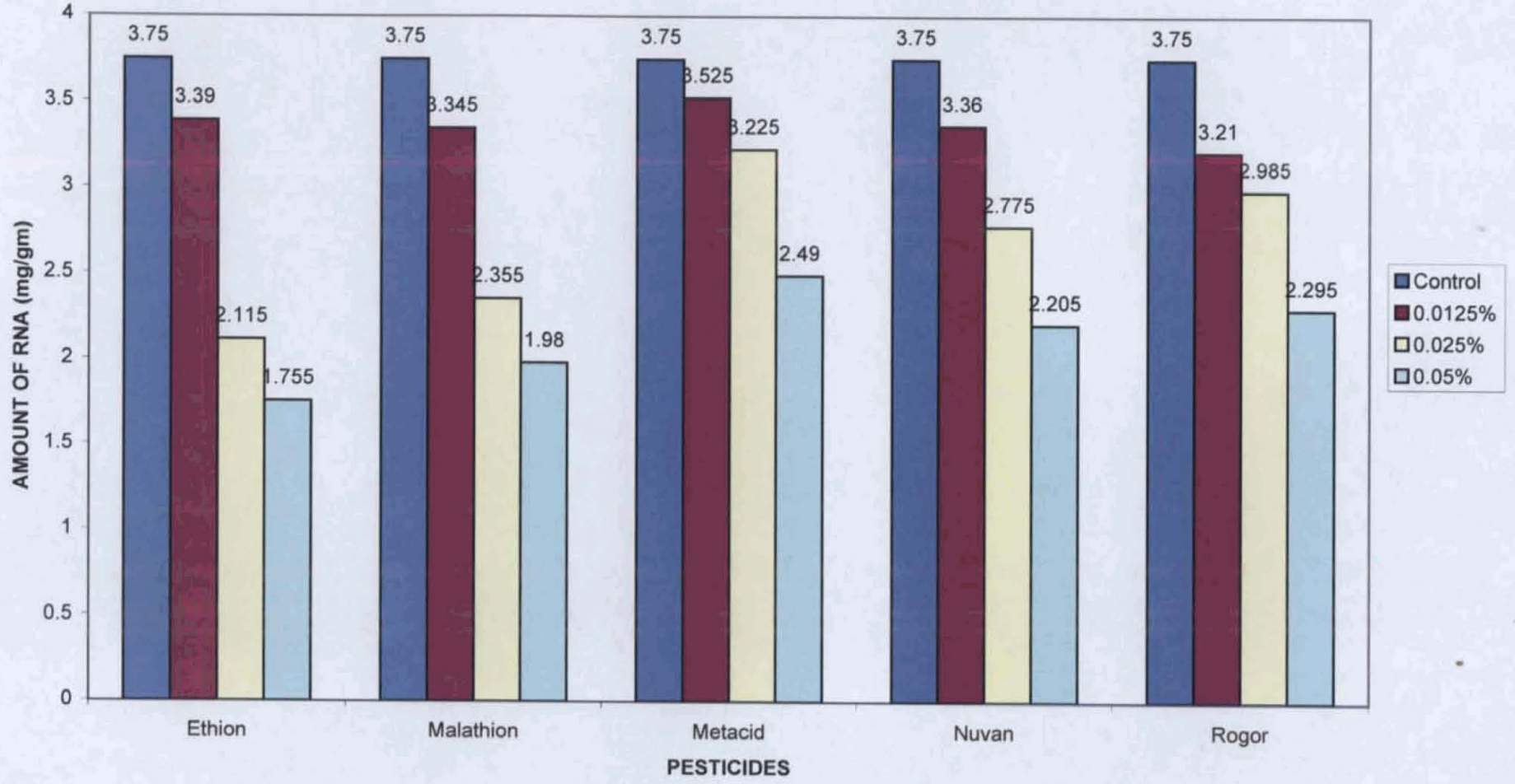


FIG. 133. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON THE AMOUNT OF BUFFER SOLUBLE PROTEIN IN ROOT TIP CELLS OF *ALLIUM CEPA* AFTER 1 HOUR TREATMENT

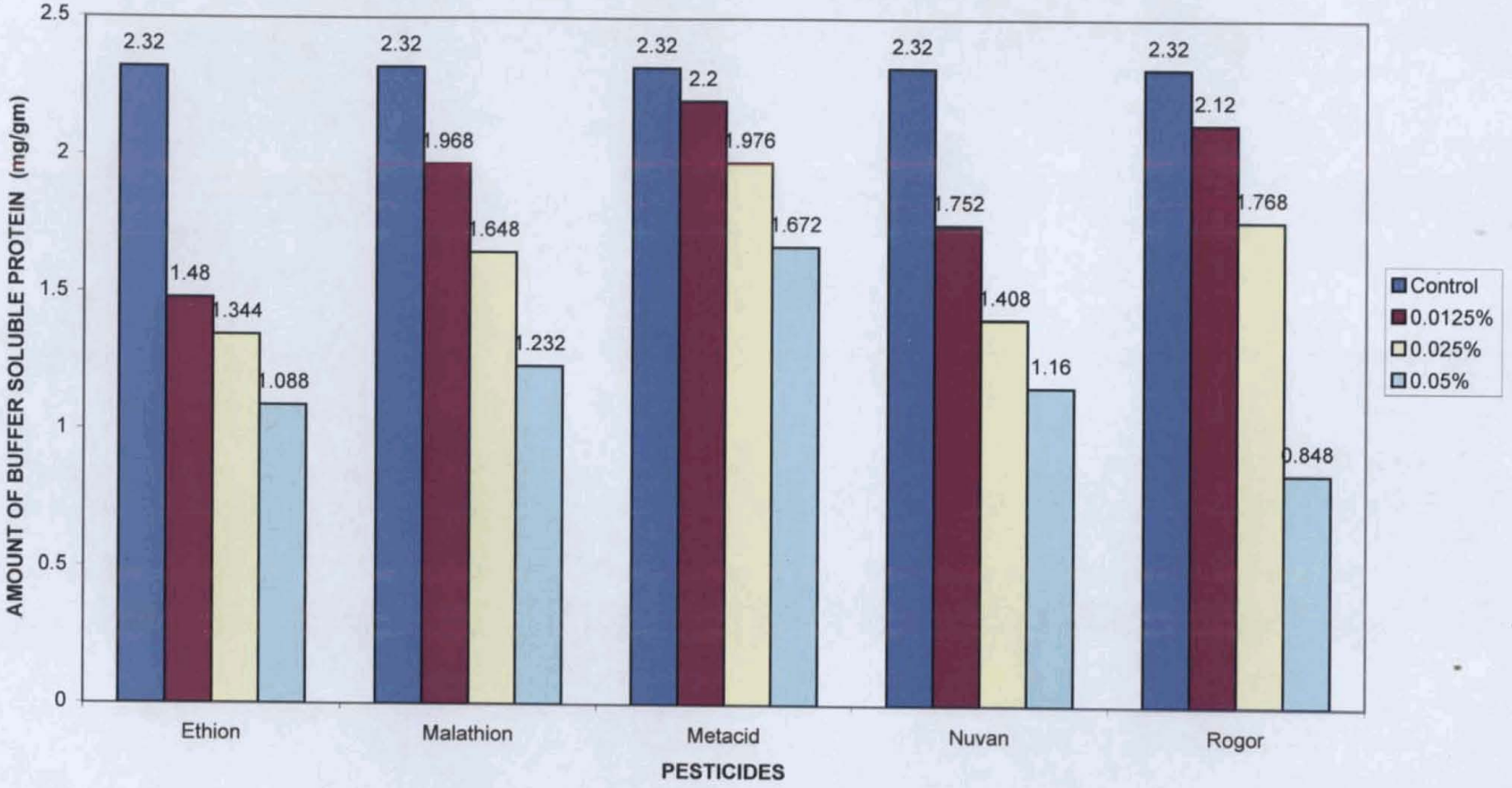


FIG. 134. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON THE AMOUNT OF BUFFER SOLUBLE PROTEIN IN ROOT TIP CELLS OF *ALLIUM CEPA* AFTER 2 HOURS OF TREATMENT

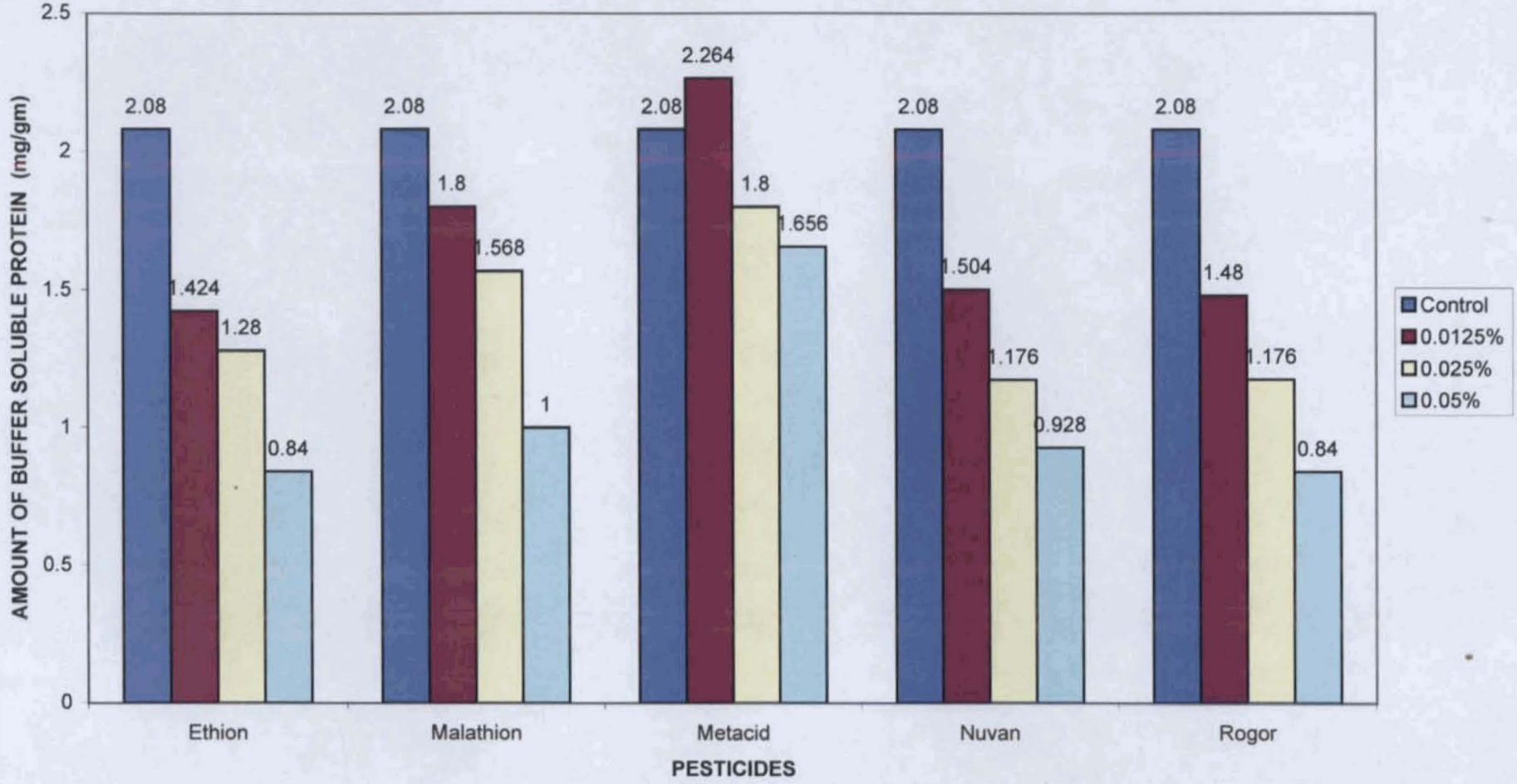


FIG. 135. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON THE AMOUNT OF BUFFER SOLUBLE PROTEIN IN ROOT TIP CELLS OF *ALLIUM SATIVUM* AFTER 1 HOUR TREATMENT

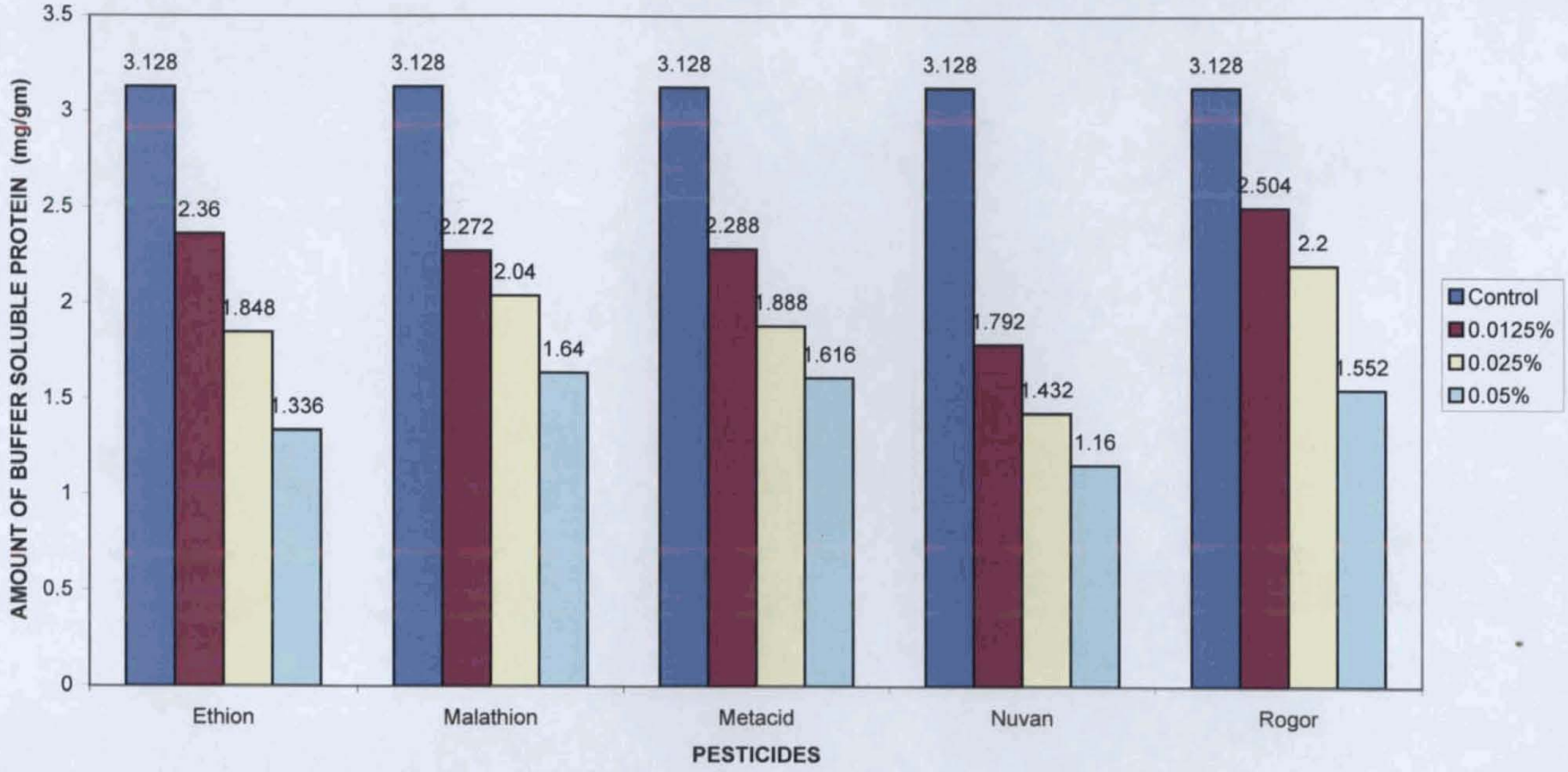


FIG. 136. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON THE AMOUNT OF BUFFER SOLUBLE PROTEIN IN ROOT TIP CELLS OF *ALLIUM SATIVUM* AFTER 2 HOURS OF TREATMENT

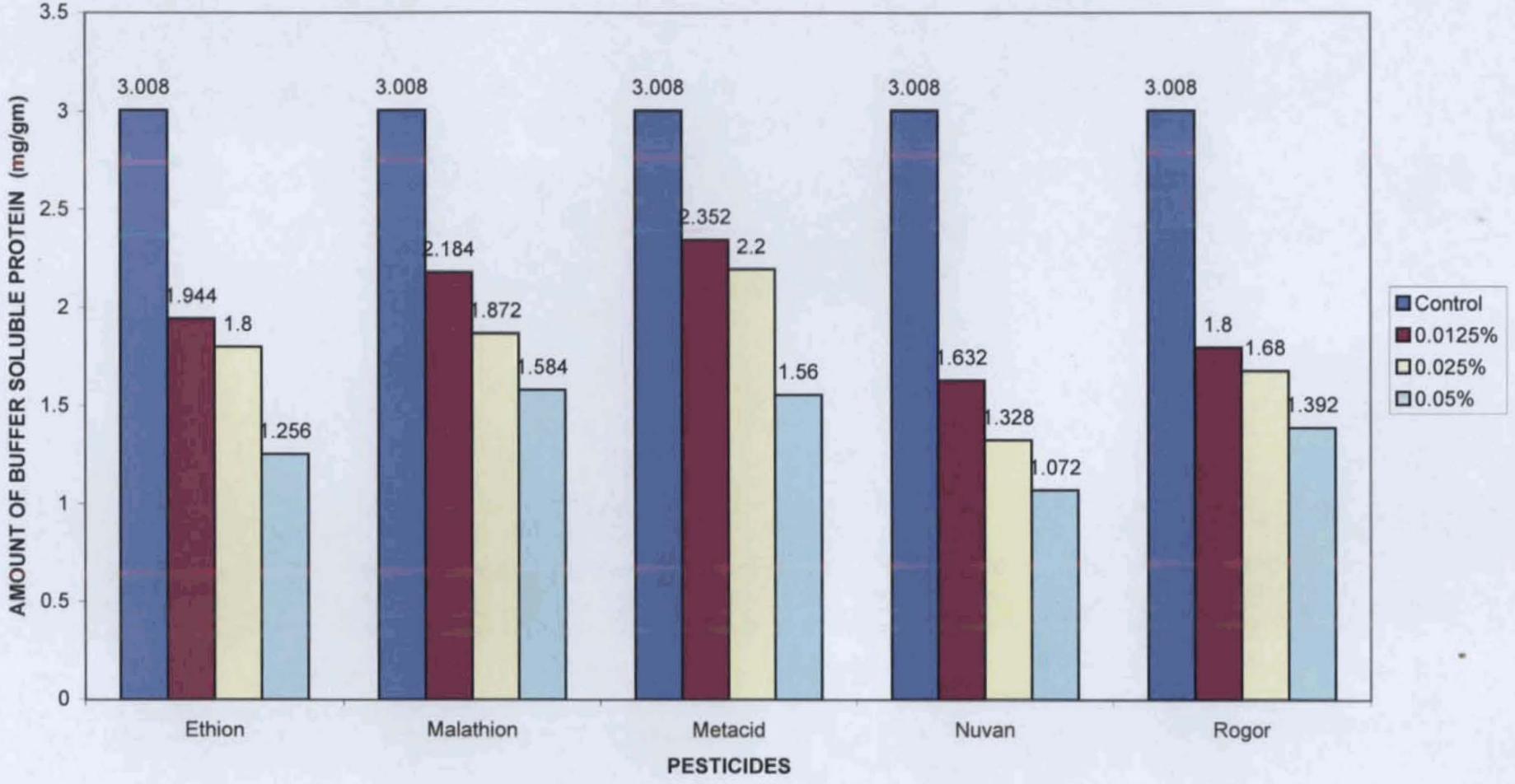


FIG. 137.COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON THE AMOUNT OF TOTAL FREE AMINOACIDS IN ROOT TIP CELLS OF *ALLIUM CEPA* AFTER 1 HOUR TREATMENT

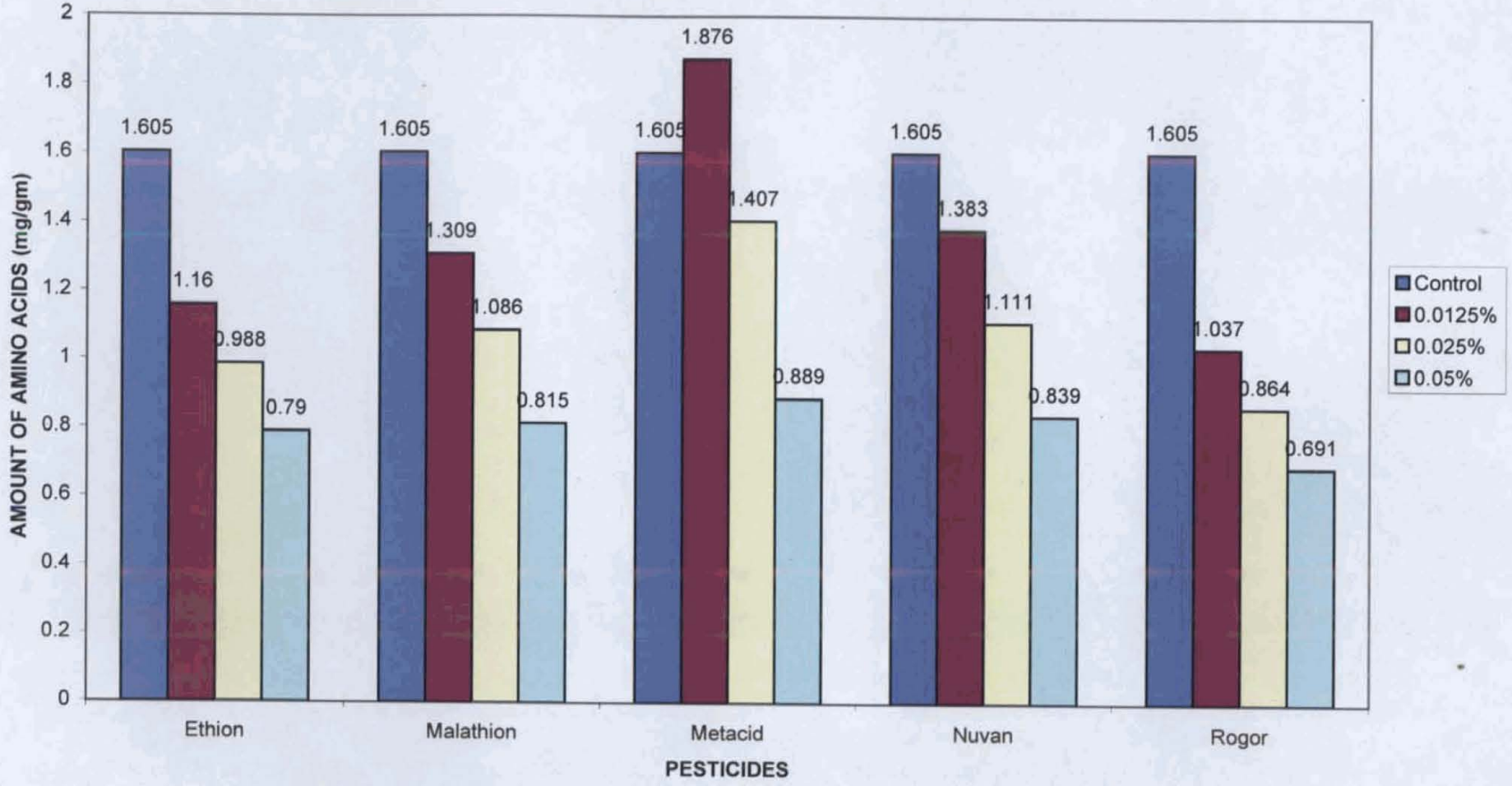


FIG. 138. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON THE AMOUNT OF TOTAL FREE AMINOACIDS IN ROOT TIP CELLS OF *ALLIUM CEPA* AFTER 2 HOURS OF TREATMENT

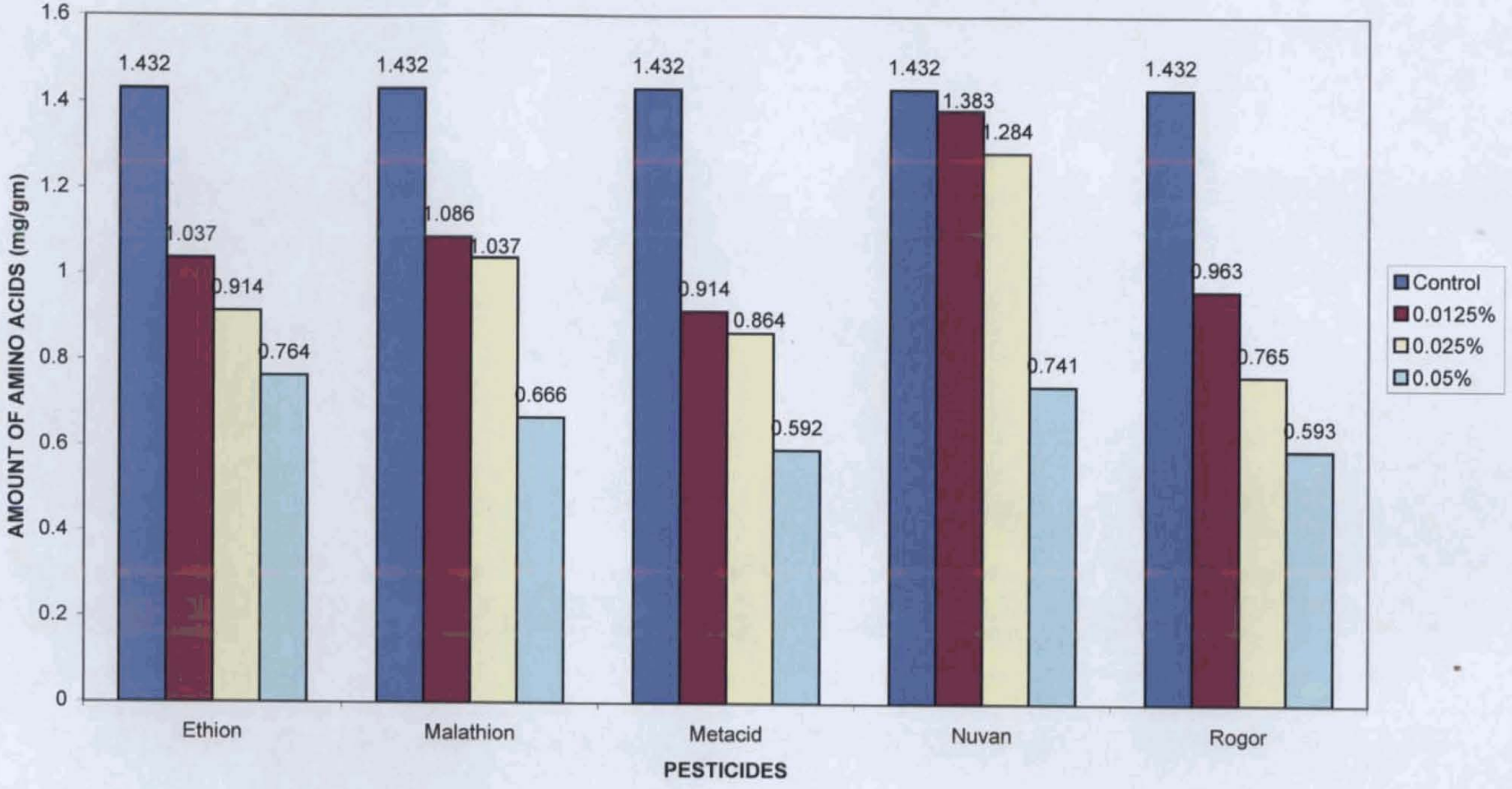


FIG. 139. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON THE AMOUNT OF TOTAL FREE AMINOACIDS IN ROOT TIP CELLS OF *ALLIUM SATIVUM* AFTER 1 HOUR TREATMENT

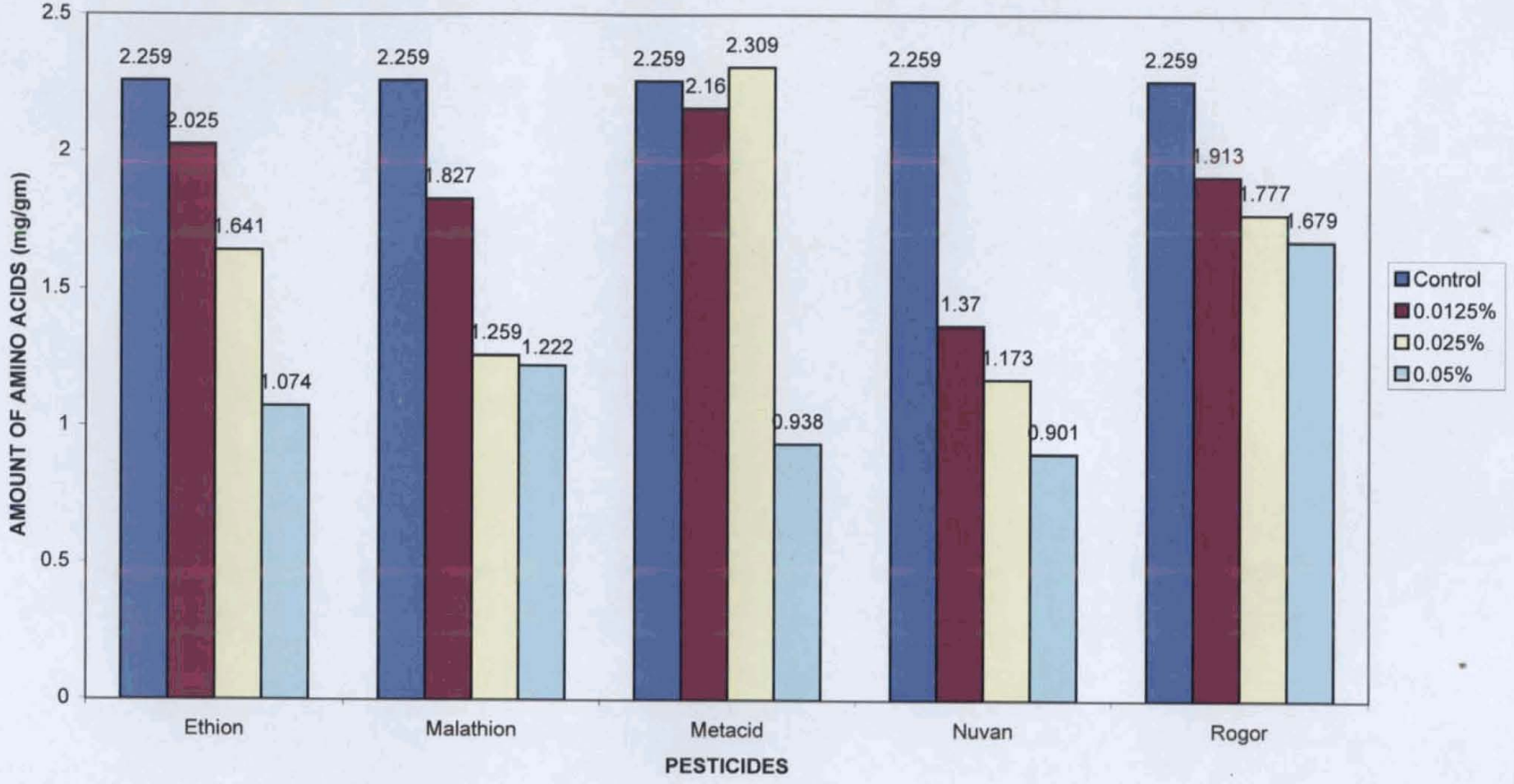
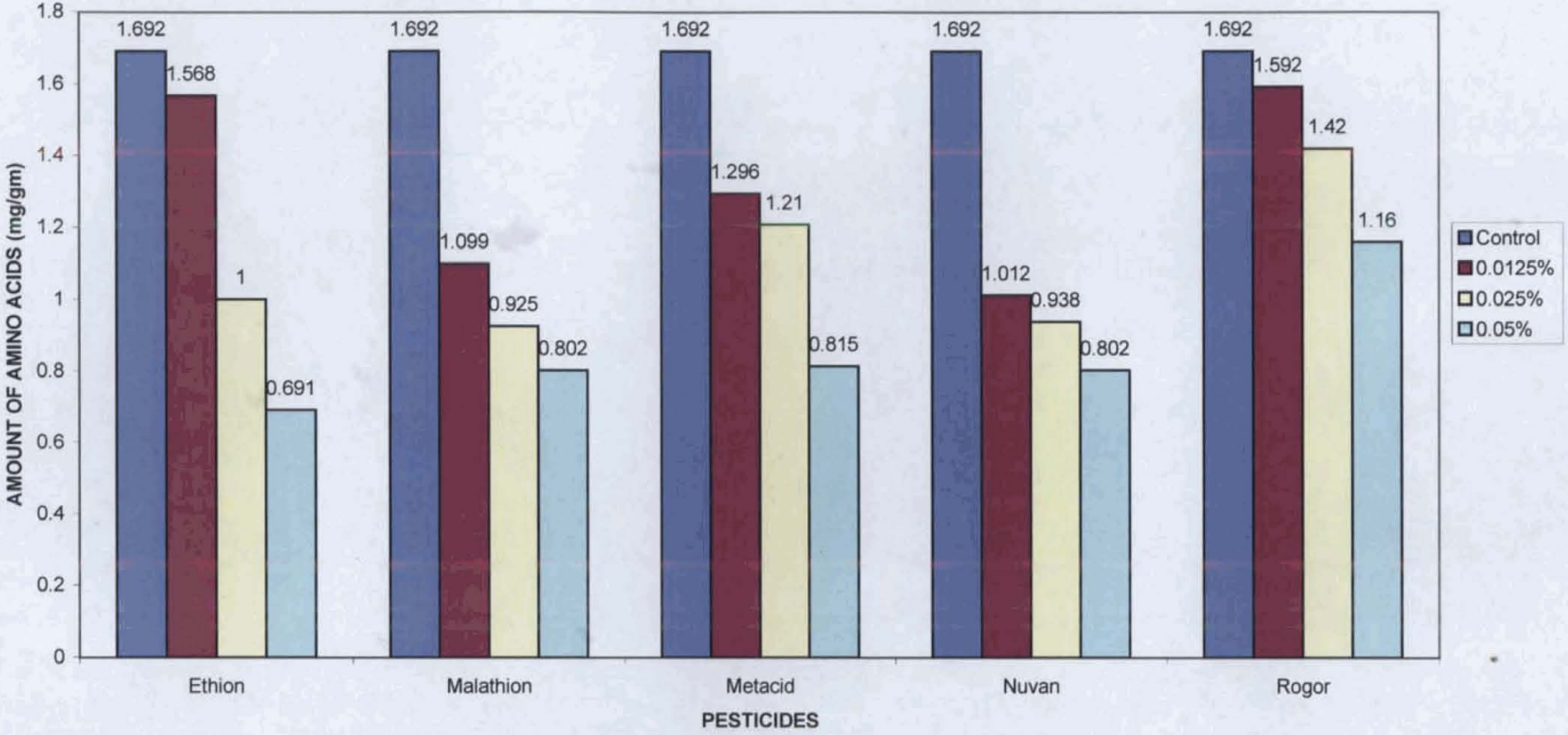
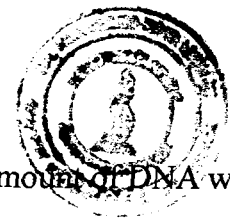


FIG. 140. COMPARATIVE EFFECTS OF PESTICIDES ETHION, MALATHION, METACID, NUVAN AND ROGOR ON THE AMOUNT OF TOTAL FREE AMINO ACIDS IN ROOT TIP CELLS OF *ALLIUM SATIVUM* AFTER 2 HOURS OF TREATMENT





expressed variation in their amounts of DNA. The amount of DNA were 4.368 ± 0.048mg/g, 4.032 ± 0.016mg/g and 3.792 ± 0.032mg/g fresh weights for 0.0125%, 0.025% and 0.05% of Nuvan respectively after one-hour treatment (Fig.125). In two–hours treatment the amount of DNA were 4.336 ± 0.016 mg/g, 3.76 ± 0.016 mg/g and 3.36 ± 0.032 mg/g fresh weight respectively (Fig.126). All these estimations were tabulated in table 17. The concentrations of pesticide and the amount of DNA were inversely proportional to each other.

8. Estimation of DNA after the treatment of *Allium sativum* root tip cells with different concentrations of Nuvan: -

The amount of DNA was estimated after the treatment of *A. sativum* root tip cells with different concentrations of the pesticide Nuvan. The DNA content in the various treatments with *A. sativum* was found to be less than that of the controls. Normally the DNA contents were found to decrease with the increasing concentrations of the pesticide Nuvan. DNA content ranges from 3.936 ± 0.032 mg/g to 2.848 ± 0.016mg/g after one hour treatment (Fig.127) and from 4.576 ± 0.048mg/g to 3.12 ± 0.032mg/g (Fig.128). The amount of DNA detected in each treatment concentrations and time durations, are depicted in table 18.

9. Estimation of DNA after the treatment of *Allium cepa* root tip cells with different concentrations of Rogor: -

The results observed from root tips exposed to various concentrations of Rogor during one and two-hours treatments are summarized in the table 19. The amount of DNA decreased with the increase in insecticide concentration. DNA quantified in one-hour treatment showed 4.384 ± 0.032 mg/g, 4.160 ± 0.016mg/g and 2.448 ± 0.032 mg/g fresh weight for the 0.0125%, 0.025% and 0.05% of insecticide treated materials respectively (Fig.125). The two-hours treated materials of *A. cepa* quantified 4.112 ± 0.032 mg/g, 3.136 ± 0.032mg/g, 2.448 ±

0.016 mg/g fresh weight from the different concentrations such as 0.0125%, 0.025% and 0.05% of pesticide respectively (Fig.126).

10. Esimation of DNA after the treatment of *Allium sativum* root tip cells with different concentrations of Rogor: -

Table 20 reveals the inhibition of DNA synthesis by the pesticide Rogor on *A. sativum* in two different time durations. The results showed a decrease in the amount of DNA which range from 5.232 ± 0.016 mg/g to 3.232 ± 0.016mg/g fresh weight after one-hour treatment (Fig.127). The same trend was observed in the two-hours treatment, where the amount of DNA ranges from 5.008 ± 0.016 mg/g to 2.016 ± 0.032 mg/g fresh weight (Fig.128). The content of DNA was negatively affected when the concentrations were increased and the time was prolonged.

II. EFFECT ON RNA

1. Estimation of RNA after the treatment of *Allium cepa* root tip cells with different concentrations of Ethion: -

The influence of pesticide Ethion on RNA synthesis was determined and is reported in table 21. The RNA content decreased with increasing concentrations and there was a maximum inhibition at 0.05% treatment in both time durations. The amount of RNA estimated at different concentrations of pesticide such as 0.0125%, 0.025% and 0.05% were 1.776 ± 0.012 mg/g, 1.584 ± 0.006 mg/g and 1.356 ± 0.006 mg/g fresh weight at one-hour treatment (Fig.129) and 1.716 ± 0.012 mg/g, 1.104 ± 0.006 mg/g and 0.918 ± 0.006 mg/g fresh weight at two hour treatment respectively (Fig.130).

2. Estimation of RNA after the treatment of *Allium sativum* root tip cells with different concentrations of Ethion: -

The effect of Ethion on root tip cells of *A. sativum* was evaluated. The control materials of *A. sativum* have more amounts of RNA than the controls of

A. cepa. The detected amounts of RNA are depicted in table 22. The amount of RNA was found to be 3.990 ± 0.015 mg/g, 3.675 ± 0.030 mg/g and 3.405 ± 0.030 mg/g fresh weight after one-hour treatment in 0.0125%, 0.025% and 0.05% of Ethion respectively (Fig.131) and 3.390 ± 0.045 mg/g, 2.115 ± 0.015 mg/g and 1.755 ± 0.030 mg/gram fresh weight after treatment in 0.0125%, 0.025% and 0.05% of Ethion at two-hours duration (Fig.132).

3. Estimation of RNA after the treatment of *Allium cepa* root tip cells with different concentrations of Malathion: -

The inhibitory effects of different concentrations of Malathion on root tip cells of *A. cepa* are listed in table 23. In all the cases, the inhibition of RNA increased with increase in percentage of pesticide concentrations. The amount of RNA ranges from 1.602 ± 0.006 mg/g to 0.93 ± 0.012 mg/g fresh weight after one hour treatment (Fig.129) and from 1.254 ± 0.006 mg/g to 0.63 ± 0.018 mg/g fresh weight after two-hours treatment (Fig.130). In all treatments within each pesticide concentrations and time durations, the RNA contents were found to be less than those of the controls.

4. Estimation of RNA after the treatment of *Allium sativum* root tip cells with different concentrations of Malathion: -

The effects of pesticide Malathion on RNA content in *A. sativum* was observed. The results revealed that there was a considerable decrease of RNA content with the higher dose treatments of pesticide. The results are shown in the table 24. The treatments caused gradual decrease in the RNA amount during one-hour treatment, which ranges from 3.825 ± 0.030 mg/g to 2.04 ± 0.045 mg/g fresh weight (Fig.131). The same trend was found in the two-hour treatment as the concentrations and time prolonged. Here it range from 3.345 ± 0.030 mg/g to 1.98 ± 0.015 mg/g fresh weight (Fig.132).

5.Estimation of RNA after the treatment of *Allium cepa* root tip cells with different concentrations of Metacid: -

The amount of RNA was calculated after the treatment of *A. cepa* root tip cells with different concentrations of pesticide Metacid in two time durations. The amount of RNA was 2.382 ± 0.12 mg/g, 1.902 ± 0.012 mg/g and 1.584 ± 0.018mg/g fresh weights for 0.0125%, 0.025% and 0.05% of Metacid respectively after one-hour treatment (Fig.129). In two-hours treatment the amount of RNA were 1.704 ± 0.018 mg/g, 1.524 ± 0.006 mg/g and 0.888 ± 0.006 mg/g fresh weight respectively (Fig.130). Metacid has an inhibitory effect on RNA synthesis and it was found that inhibition increased with increased dose of pesticide. The results obtained are tabulated in the table 25.

6. Estimation of RNA after the treatment of *Allium sativum* root tip cells with different concentrations of Metacid: -

Table 26 gives the total RNA content in controls and different dose levels in two time durations. In general, the amount of RNA was decreased as the concentration of pesticide was increased during all the treatment. The results reveal that the increased time of treatment was of some influence on synthesis of RNA. The amount of RNA ranges from 3.81 ± 0.045mg/g to 2.46 ± 0.045 mg/g fresh weight after one-hour treatment (Fig.131) and from 3.525 ± 0.060 mg/g to 2.49 ± 0.030 mg/g fresh weight after two-hours treatment (Fig.132).

7. Estimation of RNA after the treatment of *Allium cepa* root tip cells with different concentrations of Nuvan: -

Data on RNA content obtained after the treatment of *A. cepa* root tip cells with different concentrations of Nuvan in two different time durations showed a negative correlation. The results are recorded in table 27. The amount of RNA content measured was considerably lesser than those found in the controls and there was a decrease in the RNA content with increase of pesticide

concentrations and time durations. The RNA content was found to range from 2.37± 0.012 mg/g to 1.176 ± 0.006 mg/g fresh weight after one-hour treatment (Fig.129) and from 1.374 ± 0.006 mg/g to 0.75 ± 0.018 mg/g fresh weight after two-hour treatment (Fig.130).

8. Estimation of RNA after the treatment of *Allium sativum* root tip cells with different concentrations of Nuvan: -

The total RNA content in *A. sativum* after pesticide treatment was evaluated and tabulated in the table 28. In all the cases, the RNA content decreased with the increase in dose treatment. RNA contents were lower than those of the controls. The materials treated with 0.0125%, 0.025%, and 0.05% of pesticide concentrations for one-hour exhibited 3.540 ± 0.015mg/g, 2.910 ± 0.030mg/g and 2.340 ± 0.015mg/g fresh weight of RNA respectively (Fig.131). The materials treated with the same concentrations after two-hours measured 3.360 ± 0.060mg/g, 2.775 ± 0.060mg/g and 2.205 ± 0.030mg/g fresh weight of RNA respectively (Fig.132). It was found that the effects were dependent on time and concentrations.

9. Estimation of RNA after the treatment of *Allium cepa* root tip cells with different concentrations of Rogor: -

The amount of RNA was quantitatively estimated and is given in the table 29. The results showed considerable decrease with the increase of dose treatments of the pesticide Rogor. Increased time of treatment was of some influence on synthesis of RNA. Within each pesticide treatment, the RNA contents were lower than those of the controls. The amount of RNA ranges from 2.064 ± 0.012 mg/g to 1.242 ± 0.006 mg/g fresh weight after one-hour treatment (Fig.129) and from 1.668 ± 0.006 mg/g to 0.768 ± 0.006 mg/g fresh weight after two-hours treatment (Fig.130).

10. Estimation of RNA after the treatment of *Allium sativum* root tip cells with different concentrations of Rogor: -

The effect of Rogor on root tip cells of *Allium sativum* was noted and the amount of RNA measured were summarized in table 30. The amount of RNA ranges from 3.69 ± 0.054 mg/g to 2.655 ± 0.030 mg/g fresh weight after one-hour treatment (Fig.131) and from 3.21 ± 0.045 mg/g to 2.295 ± 0.030 mg/g fresh weight after two-hours treatment (Fig.132). The amount of RNA was decreased as the concentration of pesticide was increased at all treatment periods. There was a maximum inhibition at 0.05% treatment in both time durations. There exists a negative correlation between the RNA content and concentration of pesticide.

III. EFFECT ON BUFFER SOLUBLE PROTEIN.

1. Estimation of buffer soluble protein after the treatment of *Allium cepa* root tip cells with different concentrations of Ethion: -

The amount of buffer soluble protein was calculated after root tip cells were treated with different concentrations of Ethion and are presented in the table 31. The amount of buffer soluble protein in the control material kept in distilled water for one-hour and two-hours were 2.320 ± 0.016mg/g and 2.080 ± 0.008mg/g fresh weights respectively. The amount of buffer soluble protein ranges from 1.48 ± 0.016mg/g to 1.088 ± 0.016 mg/g fresh weight after one-hour treatment (Fig.133) and from 1.424 ± 0.008 mg/g to 0.840 ± 0.024 mg/g fresh weight after two-hours treatment (Fig.134). Profound decrease in the amounts could be clearly noticed in the treated materials.

2. Estimation of buffer soluble protein after the treatment of *Allium sativum* root tip cells with different concentrations of Ethion: -

Buffer soluble protein was estimated after the treatment of *A. sativum* root tip cells with different concentrations of Ethion. The protein contents measured

were 2.360 ± 0.008mg/g, 1.848 ± 0.008mg/g and 1.336 ± 0.016mg/g after treatment with 0.0125%, 0.025% and 0.05% of pesticide for one-hour (Fig.135) and 1.944 ± 0.008mg/g, 1.800 ± 0.016mg/g and 1.256 ± 0.008mg/g after treatment with 0.0125%, 0.025% and 0.05% of Ethion for two-hours (Fig.136). Controls and treated materials in both the time durations differ remarkably in their buffer soluble protein contents. It is evident from the table that the buffer soluble protein content decreased considerably as the concentration of pesticide was increased during all the treatment periods (Table 32).

3. Estimation of buffer soluble protein after the treatment of *Allium cepa* root tip cells with different concentrations of Malathion: -

The quantities of buffer soluble proteins of the treated samples of *A. cepa* are given in the table 33. The results showed a clear decrease in the amount of buffer soluble protein. When the root tips are subjected to 0.0125%, 0.025%, and 0.05% of Malathion in one-hour, the protein content lowers to 1.968 ± 0.024mg/g, 1.648 ± 0.024mg/g, 1.232 ± 0.024mg/g fresh weight respectively (Fig.133). When the root tips were treated with different percentages of Malathion, such as 0.0125%, 0.025% and 0.05% of pesticide for two-hours, the protein content became decreased to 1.800 ± 0.008mg/g, 1.568 ± 0.008mg/g and 1.000 ± 0.024mg/g fresh weight respectively (Fig.134). There was a significant decrease of protein content in both time durations.

4. Estimation of buffer soluble protein after the treatment of *Allium sativum* root tip cells with different concentrations of Malathion.

The amount of buffer soluble protein is calculated after the treatment of *A. sativum* root meristem with different concentrations of the pesticide Malathion. The results showed that there was considerable decrease in the amount of buffer soluble protein due to the effect of Malathion. The amount of buffer soluble protein ranges from 2.272 ± 0.032 mg/g to 1.64 ± 0.016 mg/g fresh weight after

one-hour treatment (Fig.135) and from 2.184 ± 0.008 mg/g to 1.584 ± 0.008 mg/g fresh weight after two-hours treatment (Fig.136). The details are shown in table 34.

5. Estimation of buffer soluble protein after the treatment of *Allium cepa* root tip cells with different concentrations of Metacid: -

Remarkable variations in the amounts of buffer soluble protein are noted among the pesticide treated samples. In most of the cases, samples treated with higher concentrations of pesticide revealed less amount of buffer soluble protein than control treated with distilled water. However, an increase in the buffer soluble protein content was shown by the 0.0125% pesticide treated samples after two-hours treatment. The estimated amount of protein ranges from 2.2 ± 0.016mg/g to 1.672 ± 0.008mg/g fresh weights after one-hour treatment (Fig.133) and from 2.264 ± 0.016mg/g to 1.656 ± 0.016mg/g fresh weights after two-hours treatment (Fig.134). All these datas are summarized in the table 35. Increased time of treatment shows some influence on synthesis of buffer soluble protein.

6. Estimation of buffer soluble protein after the treatment of *Allium sativum* root tip cells with different concentrations of Metacid: -

Buffer soluble protein was quantified after the treatment of *A. sativum* root tip cells with different concentrations of Metacid and found that there was a decrease in the amount of buffer soluble protein as the concentration of pesticide increased. 0.0125%, 0.025% and 0.05% of pesticide treated materials of both time durations were 2.288 ± 0.008mg/g, 1.888 ± 0.008mg/g, 1.616 ± 0.008mg/g (Fig.135) and 2.352 ± 0.032mg/g, 2.200 ± 0.016mg/g, 1.560 ± 0.016mg/g fresh weight (Fig.136) respectively. Table 36 shows the details obtained after each treatment in two-time durations. There was also a negative correlation between the amount of buffer soluble protein and concentration of pesticide used.

7. Estimation of buffer soluble protein after the treatment of *Allium cepa* root tip cells with different concentrations of Nuvan: -

The buffer soluble protein contents measured were 2.320 ± 0.016mg/g and 2.080 ± 0.008mg/g for controls in two different time durations. The buffer soluble protein content ranges from 1.752 ± 0.008mg/g to 1.16 ± 0.024mg/g fresh weight after one-hour treatment (Fig.133) and from 1.504 ± 0.008mg/g to 0.928 ± 0.016mg/g fresh weights after two-hours treatment (Fig.134). The influence of pesticide on buffer soluble protein content was prominent as the concentrations of pesticide increased at all treatment periods. The details are shown in table 37.

8. The estimation of buffer soluble protein after the treatment of *Allium sativum* root tip cells with different concentrations of Nuvan: -

The effects of different concentrations of Nuvan on buffer soluble protein content after the treatments of *A. sativum* root tip cells are depicted in table 38. Controls of both the time durations possess maximum amount of buffer soluble protein. The amount of buffer soluble proteins were 1.792 ± 0.008 mg/g, 1.432 ± 0.008 mg/g and 1.16 ± 0.016 mg/g fresh weights for the three concentrations of Nuvan respectively after one-hour treatment (Fig.135). In two-hours treatment the amount of proteins were 1.632 ± 0.024 mg/g, 1.328 ± 0.008 mg/g and 1.072 ± 0.008 mg/g fresh weight respectively (Fig.136). The results in the table show that there is considerable decrease in the content of buffer soluble protein according to the increase of concentrations.

9. Estimation of buffer soluble protein after the treatment of *Allium cepa* root tip cells with different concentrations of Rogor: -

The effect of Rogor on estimation of buffer soluble protein from pesticide treated samples of *A. cepa* was studied. A dose related decrease of amounts of buffer soluble proteins were observed. The control materials of both time

durations had maximum amount of buffer soluble protein. The amount of buffer soluble protein ranges from 2.12 ± 0.016 mg/g to 0.848 ± 0.016 mg/g fresh weight after one-hour treatment (Fig.133) and from 1.48 ± 0.024 mg/g to 0.84 ± 0.024 mg/g fresh weight after two-hours treatment (Fig.134). Rogor caused a decrease of buffer soluble protein content at a relatively high dose level and is depicted in table 39.

10. Estimation of buffer soluble protein after the treatment of *Allium sativum* root tip cells with different concentrations of Rogor: -

Buffer soluble protein estimations of the pesticide treated samples of *A. sativum* are given in the table 40. The depicted results showed a clear decrease in the amount of protein content. When the root tips were treated with 0.0125%, 0.025% and 0.05% of Rogor for one-hour, the protein contents decreased to 2.504 ± 0.016 mg/g, 2.200 ± 0.024mg/g and 1.552 ± 0.016 mg/g fresh weight respectively (Fig.135). The reduction of buffer soluble protein content continued as the concentration increased and time prolonged. This nature was also present in the two-hours of treatment. Control was found to be with maximum protein content and when the root tips treated with different percentages of Rogor such as 0.0125%, 0.025% and 0.05%, the protein content became decreased to 1.800 ± 0.016 mg/g, 1.680 ± 0.008 mg/g, 1.392 ± 0.032 mg/g fresh weight respectively (Fig.136). There was a negative correlation between concentration of pesticide and the content of buffer soluble protein.

IV. EFFECT ON TOTAL FREE AMINO ACIDS

1. Estimation of total free amino acids after the treatment of *Allium cepa* root tip cells with different concentrations of Ethion: -

The amount of total free amino acids are calculated after the treatment of *A. cepa* root tip cells with different concentrations of Ethion and are presented in table 41. The amount of total free amino acids present in the control materials

kept in distilled water for one-hour and two-hours were 1.605 ± 0.075 mg/g and 1.432 ± 0.025 mg/g fresh weight respectively. The amount of total free amino acids range from 1.16 ± 0.025 mg/g to 0.79 ± 0.075 mg/g fresh weight after one-hour treatment (Fig. 137) and from 1.037 ± 0.075 mg/g to 0.764 ± 0.050 mg/g fresh weight after two-hours treatment (Fig.138). A reduction in the amount of total free amino acids could be clearly noticed in the treated materials.

2. Estimation of total free amino acids after the treatment of *Allium sativum* root tip cells with different concentrations of Ethion: -

Total free amino acids were estimated after the treatment of *A. sativum* root tip cells with different concentrations of Ethion. It has been recorded that the total free amino acid content is comparatively more in the control materials than treated root tips. The amino acid content measured were 2.025 ± 0.050 mg/g, 1.641 ± 0.013mg/g, 1.074 ± 0.013 mg/g fresh weight for 0.0125%, 0.025% and 0.05% pesticide treated materials at one-hour (Fig.139) and 1.568 ± 0.013mg/g, 1.000 ± 0.013mg/g, and 0.691 ± 0.025mg/g fresh weight for 0.0125%, 0.025% and 0.05% Ethion treated materials at two-hours (Fig.140). Control and treated materials in both the time durations differ remarkably in their total free amino acid content. It was evident from the table that the total free amino acid content decreased considerably as the concentration of pesticide was increased at all treatment periods (Table 42).

3. Estimation of total free amino acids after the treatment of *Allium cepa* root tip cells with different concentrations of Malathion: -

The quantities of total free amino acids of the pesticide treated samples of *A. cepa* are given in the table 43. The results showed a clear decrease in the amount of total free amino acids. When the root tips were subjected to 0.0125%, 0.025%, 0.05% of Malathion in one-hour, the total free amino acid content lowers to 1.309 ± 0.125 mg/g, 1.086 ± 0.050 mg/g, 0.815 ± 0.075 mg/g fresh

weight respectively (Fig.137). The reduction of total free amino acid content continued as the concentration increased. This nature was also present in the two-hour treatment. When the root tips were treated with different percentages of Malathion such as 0.0125%, 0.025%, 0.05%, the total free amino acid content became decreased to 1.086 ± 0.025mg/g, 1.037 ± 0.150mg/g and 0.666 ± 0.075mg/g fresh weights respectively (Fig.138).

4. Estimation of total free amino acids after the treatment of *Allium sativum* root tip cells with different concentrations of Malathion: -

The amount of total free amino acids was calculated after the treatment of *A. sativum* root meristem with different concentrations of the pesticide Malathion. The amount of total free amino acids ranges from 1.827 ± 0.013 mg/g to 1.222 ± 0.037 mg/g fresh weight after one-hour treatment (Fig.139) and from 1.099 ± 0.037 mg/g to 0.802 ± 0.025 mg/g fresh weight after two-hours treatment (Fig.140). The results showed that there were considerable decreases in their amount due to the effect of Malathion. The details of it are shown in table 44.

5. Estimation of total free amino acids after the treatment of *Allium cepa* root tip cells with different concentrations of Metacid: -

Remarkable variations in the amount of total free amino acids are noted among the pesticide treated samples. In certain cases, however samples treated with higher concentrations of pesticide revealed lesser amounts of total free amino acids than controls treated with distilled water. The materials treated with 0.0125%, 0.025% and 0.05% of Metacid at one-hour exhibits 1.876 ± 0.075mg/g, 1.407 ± 0.025mg/g, 0.889 ± 0.025mg/g (Fig.137) and at two hours exhibit 0.914 ± 0.125mg/g, 0.864 ± 0.050mg/g, 0.592 ± 0.100mg/g fresh weight respectively (Fig.138). When the root tips were treated with 0.0125% of pesticide Metacid at one-hour, there was a slight increase in the amount of total free amino acids. All these datas are summarized in table 45. Generally, the amount of total free amino

acid decreased after each treatment with different concentrations of pesticide Metacid as the concentrations of pesticide was increased at all treatment periods except 0.0125% of pesticide at one-hour. Increased time of treatment was of some influence on synthesis of total free amino acids.

6. Estimation of total free amino acids after the treatment of *Allium sativum* root tip cells with different concentrations of Metacid: -

Total free amino acids were quantified after the treatment of *A. sativum* root tip cells with different concentrations of Metacid and found that there was a decrease in the amount of total free amino acid as the concentration of pesticide increased. When the root tips were treated with 0.0125%, 0.025% and 0.05% of pesticide in both time durations, the amount of total free amino acids were 2.160 ± 0.013mg/g, 2.309 ± 0.013 mg/g and 0.938 ± 0.039mg/g fresh weight for one-hour (Fig.139) and 1.296 ± 0.013mg/g, 1.210 ± 0.013mg/g and 0.815 ± 0.039mg/g fresh weight for two-hours treatment (Fig.140). Table 46 shows the details obtained after each treatment in two time durations. There was also a negative correlation between the amount of total free amino acids and concentrations of pesticide used. The root tips treated with 0.025% of pesticide for one-hour exhibits slight increase in the amount of total free amino acid content than the control.

7. Estimation of total free amino acids after the treatment of *Allium cepa* root tip cells with different concentrations of Nuvan: -

The total free amino acids measured were 1.605 ± 0.075mg/g and 1.432 ± 0.025mg/g fresh weight for controls for one-hour and two-hours treatment durations. The amount of total free amino acid ranges from 1.383 ± 0.025 mg/g to 0.839 ± 0.025 mg/g fresh weight after one-hour treatment (Fig.137) and from 1.383 ± 0.050 mg/g to 0.741 ± 0.50 mg/g fresh weight after two-hours treatment (Fig.138). The influence of pesticide Nuvan on total free amino acids content

was increased as the concentrations of pesticide increased at all treatment periods. The details are shown in table 47.

8. Estimation of total free amino acids after the treatment of *Allium sativum* root tip cells with different concentrations of Nuvan: -

The effects of different concentrations of Nuvan on total free amino acid content in root tips of *A. sativum* are depicted in the table 48. Controls of both time durations possessed maximum amount of total free amino acids. The amount of total free amino acids were 1.37 ± 0.037 mg/g, 1.173 ± 0.037 mg/g and 0.901 ± 0.025 mg/g fresh weight after one-hour treatment (Fig.139) and for two-hours treatment the amount of total free amino acids were 1.012 ± 0.025mg/g, 0.938 ± 0.025 mg/g and 0.802 ± 0.037 mg/g fresh weight (Fig.140) respectively. The results depicted in the table showed that there was considerable decrease in the amount of total free amino acids according to the increase in concentration of pesticide.

9. Estimation of total free amino acids after the treatment of *Allium cepa* root tip cells with different concentrations of Rogor: -

The effects of Rogor on the total free amino acids of pesticide treated samples of *A. cepa* were studied. A dose related decrease in the amount of total free amino acids was observed. The control materials of both time durations had maximum amount of total free amino acids. The amount of total free amino acids range from 1.037 ± 0.1 mg/g to 0.691 ± 0.025mg/g fresh weight after one-hour treatment (Fig.137) and from 0.963 ± 0.075 mg/g to 0.593 ± 0.50 mg/g fresh weight after two-hours treatment (Fig.138). Rogor caused a decrease of total free amino acid content at a relatively high dose level. In all the investigations with this pesticide, the treated materials caused a decrease of amino acid contents as compared with controls (Table 49).

10. Estimation of total free amino acids after the treatment of *Allium sativum* root tip cells with different concentrations of Rogor: -

The quantities of total free amino acids of the pesticide treated samples of *A. sativum* are given in the table 50. When the root tips were subjected to 0.0125%, 0.025% and 0.05% of Rogor in one-hour, the total free amino acid content lowered to 1.913 ± 0.025 mg/g, 1.777 ± 0.037 mg/g, 1.679 ± 0.037 mg/g fresh weight respectively (Fig.139). The reduction in their amounts continued as the concentration increased. This was also observed in the two-hours of treatment. Control was found to be with maximum total free amino acid content and when the root tips were treated with different percentages of Rogor such as 0.0125%, 0.025%, 0.05%, the total free amino acid content decreased to 1.592 ± 0.100 mg/g, 1.420 ± 0.025 mg/g, 1.160 ± 0.037 mg/g fresh weight respectively (Fig. 140). There was a significant decrease of total free amino acids in both time durations.

TABLE – 1. MITOTIC INDEX, TYPES AND NUMBERS OF MITOTIC ABNORMALITIES INDUCED BY THE DIFFERENT CONCENTRATIONS OF ETHION IN *ALLIUM CEPA* ROOT TIPS AFTER DIFFERENT TREATMENT DURATIONS

Time	Concentration of pesticide (%)	No. of cells observed	Metaphase							Anaphase					Telophase				Interphase			Frequency of abnormal cells (%)	Mitotic Index
			Normal	Diagonal and sticky	Fragmentation and partial stickiness	Stickiness and dislocation	Clumping	Ball metaphase with a lesion	Irregular	Normal	Early movement	Diagonal	Diagonal with bridges	Stickiness and bridges	Normal	Stickiness	Laggard	Multiple bridges	Nuclear extrusion	Micronuclei	Binucleate cell		
1 Hour	Control	1571	130							70					137								21.45
	0.0125	1498	110	1	1	3			4	65	4			2	127	1	1					1.14	21.29
	0.025	1507	98			3	2			57	3	3		4	135	3				2		1.33	20.57
	0.05	1565	87	3	3	5	2			64		3	1	1	61	3		1		3		1.41	14.76
	0.1	1537	62			2			1	25		3	3	3	84	4	1	4			3	1.56	12.69
2 Hours	Control	1396	116							40					125								20.13
	0.0125	1298	94	2	2	1	3			58	2		1		76	6						1.31	18.88
	0.025	1240	61	2	2	4	3			39	3			2	87	2	5	1	2	1	1	2.26	17.34
	0.05	1312	47	2	1	2	2	2		26	3	3		3	79	4			3	3	3	2.36	13.95
	0.1	1405	41	4		2	7		3	25		4		5	66	5				3	5	2.7	12.1

TABLE – 2. MITOTIC INDEX, TYPES AND NUMBERS OF MITOTIC ABNORMALITIES INDUCED BY THE DIFFERENT CONCENTRATIONS OF ETHION IN *ALLIUM SATIVUM* ROOT TIPS AFTER DIFFERENT TREATMENT DURATIONS

Time	Concentration of pesticide (%)	No. of cells observed	Metaphase							Anaphase					Telophase					Interphase		Frequency of abnormal cells (%)	Mitotic Index
			Normal	Diagonal	Sticky irregular fragments	Stickiness	C -metaphase	Misorientation	Clumping	Normal	Diagonal with early movement	Multipolarity	Bridges & early movement	Lagging chromosome fragments	Normal	Stickiness	Diagonal & sticky	Tripolarity and unequal groups	Bridge & laggard	Nuclear lesion	Binucleate cell with nuclear lesion		
1 Hour	Control	1416	178							13					96								20.27
	0.0125	1439	147	1	1		1		1	22	1	1	1		35	1		1	1			0.69	14.87
	0.025	1487	95	2		2		1		27	1		1	1	37	2	5		3	1	1	1.34	12.04
	0.05	1398	75	6		6		1		26	2	1	1		40		2				3	1.57	11.66
	0.1	1415	69	8		2				25	3		1		30	5			2	2		1.63	10.39
2 Hours	Control	1395	104							63					86								18.14
	0.0125	1422	125	1	1	2	1	1		33	1		1		46				2		1	0.77	15.12
	0.025	1388	81	2	2		1	6		30	1		1		47	2	2	1		1	1	1.44	12.82
	0.05	1429	60	2		4		2		25	2		7	2	46	2				2		1.61	10.78
	0.1	1510	58	4		4		3		20	2	2		2	55	2	2			4	3	1.85	10.66

TABLE – 3. MITOTIC INDEX, TYPES AND NUMBERS OF MITOTIC ABNORMALITIES INDUCED BY THE DIFFERENT CONCENTRATIONS OF MALATHION IN *ALLIUM CEPA* ROOT TIPS AFTER DIFFERENT TREATMENT DURATIONS

Time	Concentration of pesticide (%)	No. of cells observed	Metaphase					Anaphase									Telophase		Interphase		Frequency of abnormal cells (%)	Mitotic Index
			Normal	Stickiness and dislocation	Unequal segregation & association	Diagonal & sticky	C-metaphase	Normal	Stickiness & multiple bridges	Chromosome ring	Diagonal & early movement	Double bridges	Multipolarity, early movement & ring	Stickiness	Dislocation	Diagonal bridge	Normal	Multiple bridges	Elongated nucleus with lesion	Binucleate cell with nuclear lesions		
1 Hour	Control	1498	141					83									101					21.7
	0.0125	1476	137	2	1	1	1	50	1	2	1		1	1		1	72	1		1	0.88	18.43
	0.025	1540	118	2	1	1	1	45	1	2	1	1	2	1	1	1	70			1	0.97	16.1
	0.05	1397	97	2	2		2	33		3	1	1			2		50		2		1.07	14.67
	0.1	1410	85	3	2	1	2	30	2	1		1			1	2	44	1	1	1	1.28	12.55
2 Hours	Control	1487	127					55									114					19.91
	0.0125	1480	122	4	2			40	2	2					2	3	55	3			1.22	15.88
	0.025	1510	101	3	2		4	30	2	2		1	1		2		45	2		2	1.39	13.05
	0.05	1491	94	2	2	1	1	28	2	3	2	2	2		3		42			4	1.61	12.61
	0.1	1625	82	4	3	2		22	4	3	3	4	3			4	52	2		2	2.09	11.69

TABLE – 4. MITOTIC INDEX, TYPES AND NUMBERS OF MITOTIC ABNORMALITIES INDUCED BY THE DIFFERENT CONCENTRATIONS OF MALATHION IN *ALLIUM SATIVUM* ROOT TIPS AFTER DIFFERENT TREATMENT DURATIONS

Time	Concentration of pesticide (%)	No. of cells observed	Disturbed prophase	Metaphase							Anaphase					Telophase					Interphase			Frequency of abnormal cells (%)	Mitotic Index
				Normal	Diagonal	Oblique	Stickiness	Ball Metaphase	Clumping	Stickiness showing erosion	Normal	Tripolarity with bridges	Multiple bridges	Multipolarity	Ring chromosome	Normal	Stickiness	Diagonal & sticky	Tripolarity & laggard	Bridge	Binucleate cell	Micronuclei	Nuclear deformation		
1 Hour	Control	1417		123							71					82									19.48
	0.0125	1577	5	125	1	1		1	1	1	53	1		1		70	1	1		1	1		1	1.08	16.8
	0.025	1410		107	2	3	1		3	1	30		1	1	1	48	1	1	1				1	1.21	14.04
	0.05	1488		95	3		4		4		30		1			50	2	2		2	2			1.34	13.1
	0.1	1461		72	4	2	2		2	2	29		1			50	4	2			1	2		1.51	11.84
2 Hours	Control	1463		117							78					60									17.43
	0.0125	1525		108	2	2	2	1	1	1	30	1		1		62	1		1		1		1	0.98	14.09
	0.025	1571		88	2	2	2		2		26		2	1		65	1	2	2		2	2		1.27	12.67
	0.05	1511		77	3	1	2		2		25		3		1	62	1	1	6	2			1	1.52	12.38
	0.1	1522		71	5	3	3		2		33		2			65	5	6						1.71	12.81

TABLE – 5. MITOTIC INDEX, TYPES AND NUMBERS OF MITOTIC ABNORMALITIES INDUCED BY THE DIFFERENT CONCENTRATIONS OF METACID IN *ALLIUM CEPA* ROOT TIPS AFTER DIFFERENT TREATMENT DURATIONS

Time	Concentration of pesticide (%)	No. of cells observed	Metaphase							Anaphase							Telophase			Interphase			Frequency of abnormal cells (%)	Mitotic Index
			Normal	Stickiness	Fragmentation & partial stickiness	Diagonal C-metaphase	Star metaphase	Stickiness & dislocation	Polyploidy	Normal	Lagging fragment & ring	Stickiness and triple bridges	Sticky diagonal bridge	Early movement	Dissolution and stickiness	Stickiness	Normal	Stickiness with two micronuclei	Stickiness & double bridge	Sickle shaped nuclei	Nuclear fragments	Nuclear lesion		
1 Hour	Control	1463	147							69							112							22.42
	0.0125	1515	137	2	1	1	1	1	1	50		1			1	2	70	1	1	1		1	0.99	17.95
	0.025	1410	119	3	1		1	2	1	30		1		2			57	2	2			2	1.21	15.82
	0.05	1437	93	2		2	3	2	2	27	2	2				2	54	1	1		1		1.39	13.5
	0.1	1421	77	5		3	3	3	2	20	2	2					34	4		1	1	2	1.97	11.19
2 Hours	Control	1548	126							83							109							20.54
	0.0125	1617	98	2		1	1		2	77				5		3	95		4	2			1.24	17.93
	0.025	1683	87	5	2	3	2	3	2	62	1	1	2			1	79	2			1		1.49	15.03
	0.05	1692	81	3		3		3	2	30	2	1	3		3		68		2	2	5		1.71	12.29
	0.1	1585	72	5		4	3	3	3	28	2	3	3		2		44	2	1			2	2.08	11.17

TABLE – 6. MITOTIC INDEX, TYPES AND NUMBERS OF MITOTIC ABNORMALITIES INDUCED BY THE DIFFERENT CONCENTRATIONS OF METACID IN *ALLIUM SATIVUM* ROOT TIPS AFTER DIFFERENT TREATMENT DURATIONS

Time	Concentration of pesticide (%)	No. of cells observed	Metaphase						Anaphase									Telophase					Interphase		Frequency of abnormal cells (%)	Mitotic Index
			Normal	Diagonal	Stickiness in hyperploid cells	Stickiness	Misorientation	Clumping	Normal	Diagonal, early movement & dislocation	Diagonal & stathmo	Ring chromosome	Bridges & dislocation	Oblique star	Disturbed chromosome	Diagonal & early movement	Multiple bridges	Stickiness with bridge & fragment	Normal	Stickiness	Diagonal & sticky	Bridge	Tripolarity & unequal group	Micronuclei	Nuclear extrusion		
1 Hour	Control	1469	107						75										90								18.52
	0.0125	1544	98	1		1	1	1	50		1			1	1			1	60			1				0.58	14.05
	0.025	1537	81		1	2	1	1	46	1	1					1		2	58	1	1				1	0.85	12.88
	0.05	1521	67	3		3		3	42	2	1		1		1		1	1	56	2						1.18	12.03
	0.1	1565	60	3	3	3		3	40	1	2	2		1	1		3		55	1			2	1	1	1.73	11.63
2 Hours	Control	1437	112						60										66								16.56
	0.0125	1590	95	2			2	1	45		1	1			1	1			57	1	4					0.88	13.27
	0.025	1491	87		1	4	2	2	37		1			2		2	2		38	4				2		1.48	12.34
	0.05	1480	60		3	5	3	2	25		2		2				3		36	5		3			3	2.09	10.27
	0.1	1467	49	3	5	5		3	21	2	3	3			2	2	2	2	39	2					1	2.39	9.82

TABLE – 7. MITOTIC INDEX, TYPES AND NUMBERS OF MITOTIC ABNORMALITIES INDUCED BY THE DIFFERENT CONCENTRATIONS OF NUVAN IN *ALLIUM CEPA* ROOT TIPS AFTER DIFFERENT TREATMENT DURATIONS

Time	Concentration of pesticide (%)	No. of cells observed	Metaphase							Anaphase					Telophase					Inter-phase	Frequency of abnormal cells (%)	Mitotic Index
			Normal	Stickiness and dislocation	Clumping	Ball metaphase with lesion	Irregular	C-Metaphase	Misorientation	Normal	Bridges and misorientation	Erosion	Dislocation	Stickiness, bridges and ring	Normal	Misorientation & stickiness	Diagonal	Parallel bridges	Stickiness and micronuclei	Binucleate cell		
1 Hour	Control	1427	117							89					113							22.35
	0.0125	1565	113	2	2	1				80	1		2		86		5			2	0.96	18.79
	0.025	1491	98	4		3	2		2	50	1	3			70	3		1			1.27	15.9
	0.05	1514	75	5	1	3		2		43		1	3	3	72			1		1	1.32	13.87
	0.1	1596	69	7	5			3	3	40	2		5	1	58	1	1		1		1.82	12.28
2 Hours	Control	1482	112							79					115							20.65
	0.0125	1417	74	3		2			1	60		2			65	2				3	0.92	14.96
	0.025	1425	68	4	2		1			45			2		50	2	1	1	1	1	1.05	12.49
	0.05	1485	63	7		4		3		38		2	1	1	45	1	1			1	1.41	11.25
	0.1	1491	56	3	5	3	3	2	2	30	1	1	1	2	37	1	2	4			2.01	10.26

TABLE – 8. MITOTIC INDEX, TYPES AND NUMBERS OF MITOTIC ABNORMALITIES INDUCED BY THE DIFFERENT CONCENTRATIONS OF NUVAN IN *ALLIUM SATIVUM* ROOT TIPS AFTER DIFFERENT TREATMENT DURATIONS

Time	Concentration of pesticide (%)	No. of cells observed	Metaphase					Anaphase								Telophase				Inter-phase	Frequency of abnormal cells (%)	Mitotic Index
			Normal	Sticky irregular fragments	Diagonal	C-metaphase	Stickiness	Normal	Bridges & early movement	Multipolarity	Early movement	Diagonal & sticky	Unequal separation misorientation and non synchronous chromosomes	Stathmo anaphase	Sticky star anaphase	Normal	Stickiness	Diagonal & sticky	Bridge	Nuclear lesion		
1 Hour	Control	1315	95					65								98						19.62
	0.0125	1487	96	1			3	50	1			2				58	1				0.54	14.26
	0.025	1410	79	1	3	1	1	40	1		2	1	1			56	2				0.92	13.33
	0.05	1535	63	2	2	1	2	37			2				1	53	2		2	1	0.98	10.94
	0.1	1560	59	4	3	2	2	32	2		2					41	3		2	1	1.35	9.81
2 Hours	Control	1413	112					50								73						16.63
	0.0125	1567	103		4	1	2	36	1		1					66	1	1			0.7	13.78
	0.025	1517	86	2			3	33	2		3		1	1	1	55	1	1	1	1	1.12	12.59
	0.05	1561	73		2		2	30	2	3	5	2	2	1	1	60	2	1			1.47	11.92
	0.1	1660	68	2	3		2	28	1	2	2		2	2		53	5		2	2	1.51	10.48

TABLE – 9. MITOTIC INDEX, TYPES AND NUMBERS OF MITOTIC ABNORMALITIES INDUCED BY THE DIFFERENT CONCENTRATIONS OF ROGOR IN *ALLIUM CEPA* ROOT TIPS AFTER DIFFERENT TREATMENT DURATIONS

Time	Concentration of pesticide (%)	No. of cells observed	Metaphase					Anaphase								Telophase				Interphase			Frequency of abnormal cells (%)	Mitotic Index
			Normal	Stickiness	Dislocation	Erosion & scattering	Pulverization	Normal	Star anaphase	Double bridge	Diagonal	Bridge & early movement	Multipolarity & unequal seggregation	Lagging & ring	Stathmo anaphase	Normal	Stickiness with micronuclei 2	Misorientation & stickiness	Stickiness & bridge	Nuclear deformation	Dumbbell shaped nucleus	Nuclear fragments		
1 Hour	Control	1458	115					83								99								20.37
	0.0125	1571	105	2		1	1	60		2	1	1			1	63	1		3				0.83	15.34
	0.025	1533	93	3	1			50		1	1	1	1			55	1	1	1	1	1		0.85	13.76
	0.05	1567	78	2		1	1	46	1	2	2	1		1		50	1	2	2	2		2	1.27	12.38
	0.1	1549	57	8			3	41	2	4	4		2	2	1	54	3		1		1	1	2.07	11.88
2 Hours	Control	1443	93					68								94								17.67
	0.0125	1618	85	2	2			44	1				1	1		77			1	1	1	1	0.68	13.41
	0.025	1591	79	4			1	40		2	4	2				60	1	1	2			1	1.13	12.38
	0.05	1560	68	4		3	2	37		3	2	2	2			57	1		1				1.28	11.67
	0.1	1517	52	6	3			30		3	8	3				34	2		4	2		1	2.11	9.76

TABLE-10. MITOTIC INDEX, TYPES AND NUMBERS OF MITOTIC ABNORMALITIES INDUCED BY THE DIFFERENT CONCENTRATIONS OF ROGOR IN *ALLIUM SATIVUM* ROOT TIPS AFTER DIFFERENT TREATMENT DURATIONS

Time	Concentration of pesticide (%)	No.of cells observed	Metaphase				Anaphase								Telophase				Interphase		Frequency of abnormal cells (%)	Mitotic Index
			Normal	Diagonal	Stickiness	Clumping	Normal	Unequal segregation	Diagonal	Stathmo anaphase	Bridge & early movement	Diagonal and early movement	Sticky bridge & deserted Chromosome	Non-synchronous movement & scattering	Tripolarity & laggard	Normal	Diagonal & sticky	Stickiness	Laggard	Binucleate cell with lesion	Micronucleus		
1 Hour	Control	1310	91				79									90							19.85
	0.0125	1435	90	1	1	1	86	1	1	1	1	1		1		90	1	1		1		0.84	19.37
	0.025	1420	80	3	3	3	45		1	1	1					40		1	1	1		1.06	12.68
	0.05	1488	77	1	1	1	30	1	1	1		2	1	1	2	50	2	3	2			1.28	11.83
	0.1	1495	65	2	2	1	29	1	3	1	2		1	1		41	3	1		1	1	1.34	10.37
2 Hours	Control	1385	95				60									80							16.97
	0.0125	1470	87	1	2	2	50	1	1	1	1	1		1		57	1	2		1		1.02	14.22
	0.025	1516	73	2	2	2	47	1	2	1	2	1		1		61	1	1		1	1	1.19	13.13
	0.05	1527	50	2	2	2	45	1	1	1	2	2	1	1		45		3		2	2	1.44	10.61
	0.1	1540	44	2	2	2	36	1	4		2	1	1	1	2	40		4		3	2	1.75	9.55

TABLE - 11. ESTIMATION OF DNA AFTER THE TREATMENT OF *ALLIUM CEPA* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF ETHION

Time	Concentrations of pesticide (%)	Amount of DNA in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	4.624	4.656	4.640 ± 0.016
	0.0125	3.696	3.664	3.680 ± 0.016
	0.025	3.600	3.632	3.616 ± 0.016
	0.05	2.480	2.448	2.464 ± 0.016
2 Hours	Control	4.608	4.480	4.544 ± 0.064
	0.0125	4.048	4.080	4.064 ± 0.016
	0.025	3.488	3.520	3.504 ± 0.016
	0.05	1.680	1.712	1.696 ± 0.016

TABLE - 12. ESTIMATION OF DNA AFTER THE TREATMENT OF *ALLIUM SATIVUM* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF ETHION

Time	Concentrations of pesticide (%)	Amount of DNA in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	5.616	5.552	5.584 ± 0.032
	0.0125	3.920	3.856	3.888 ± 0.032
	0.025	3.472	3.408	3.440 ± 0.032
	0.05	2.736	2.864	2.800 ± 0.064
2 Hours	Control	5.520	5.488	5.504 ± 0.016
	0.0125	4.592	4.624	4.608 ± 0.016
	0.025	3.760	3.696	3.728 ± 0.032
	0.05	3.120	3.184	3.152 ± 0.032

TABLE – 13. ESTIMATION OF DNA AFTER THE TREATMENT OF *ALLIUM CEPA* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF MALATHION

Time	Concentrations of pesticide (%)	Amount of DNA in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	4.624	4.656	4.640 ± 0.016
	0.0125	3.728	3.696	3.712 ± 0.016
	0.025	2.800	2.864	2.832 ± 0.032
	0.05	2.448	2.480	2.464 ± 0.016
2 Hours	Control	4.608	4.480	4.544 ± 0.064
	0.0125	3.040	3.072	3.056 ± 0.016
	0.025	2.736	2.800	2.768 ± 0.032
	0.05	2.640	2.608	2.624 ± 0.016

TABLE – 14. ESTIMATION OF DNA AFTER THE TREATMENT OF *ALLIUM SATIVUM* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF MALATHION

Time	Concentrations of pesticide (%)	Amount of DNA in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	5.616	5.552	5.584 ± 0.032
	0.0125	5.504	5.568	5.536 ± 0.032
	0.025	5.152	5.216	5.184 ± 0.032
	0.05	4.112	4.144	4.128 ± 0.016
2 Hours	Control	5.520	5.488	5.504 ± 0.016
	0.0125	5.200	5.168	5.184 ± 0.016
	0.025	4.464	4.528	4.496 ± 0.032
	0.05	3.968	3.904	3.936 ± 0.032

TABLE – 15. ESTIMATION OF DNA AFTER THE TREATMENT OF *ALLIUM CEPA* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF METACID

Time	Concentrations of pesticide (%)	Amount of DNA in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	4.624	4.656	4.640 ± 0.016
	0.0125	4.672	4.640	4.656 ± 0.016
	0.025	4.320	4.416	4.368 ± 0.048
	0.05	3.840	3.968	3.904 ± 0.064
2 Hours	Control	4.608	4.480	4.544 ± 0.064
	0.0125	4.416	4.352	4.384 ± 0.032
	0.025	4.624	4.528	4.576 ± 0.048
	0.05	4.176	4.240	4.208 ± 0.032

TABLE – 16. ESTIMATION OF DNA AFTER THE TREATMENT OF *ALLIUM SATIVUM* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF METACID

Time	Concentrations of pesticide (%)	Amount of DNA in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	5.616	5.552	5.584 ± 0.032
	0.0125	5.728	5.696	5.712 ± 0.016
	0.025	5.216	5.248	5.232 ± 0.016
	0.05	4.688	4.752	4.720 ± 0.032
2 Hours	Control	5.520	5.488	5.504 ± 0.016
	0.0125	4.144	4.240	4.192 ± 0.048
	0.025	3.760	3.696	3.728 ± 0.032
	0.05	3.568	3.664	3.616 ± 0.048

TABLE – 17. ESTIMATION OF DNA AFTER THE TREATMENT OF *ALLIUM CEPA* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF NUVAN

Time	Concentrations of pesticide (%)	Amount of DNA in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	4.624	4.656	4.64 ± 0.016
	0.0125	4.416	4.320	4.368 ± 0.048
	0.025	4.016	4.048	4.032 ± 0.016
	0.05	3.824	3.760	3.792 ± 0.032
2 Hours	Control	4.608	4.480	4.544 ± 0.064
	0.0125	4.352	4.320	4.336 ± 0.016
	0.025	3.744	3.776	3.760 ± 0.016
	0.05	3.328	3.392	3.360 ± 0.032

TABLE – 18. ESTIMATION OF DNA AFTER THE TREATMENT OF *ALLIUM SATIVUM* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF NUVAN

Time	Concentrations of pesticide (%)	Amount of DNA in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	5.616	5.552	5.584 ± 0.032
	0.0125	3.904	3.968	3.936 ± 0.032
	0.025	3.008	2.976	2.992 ± 0.016
	0.05	2.864	2.832	2.848 ± 0.016
2 Hours	Control	5.520	5.488	5.504 ± 0.016
	0.0125	4.624	4.528	4.576 ± 0.048
	0.025	3.328	3.296	3.312 ± 0.016
	0.05	3.088	3.152	3.120 ± 0.032

TABLE – 19. ESTIMATION OF DNA AFTER THE TREATMENT OF *ALLIUM CEPA* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF ROGOR

Time	Concentrations of pesticide (%)	Amount of DNA in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	4.624	4.656	4.640 ± 0.016
	0.0125	4.416	4.352	4.384 ± 0.032
	0.025	4.176	4.144	4.160 ± 0.016
	0.05	2.480	2.416	2.448 ± 0.032
2 Hours	Control	4.608	4.480	4.544 ± 0.064
	0.0125	4.080	4.144	4.112 ± 0.032
	0.025	3.104	3.168	3.136 ± 0.032
	0.05	2.464	2.432	2.448 ± 0.016

TABLE – 20. ESTIMATION OF DNA AFTER THE TREATMENT OF *ALLIUM SATIVUM* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF ROGOR

Time	Concentrations of pesticide (%)	Amount of DNA in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	5.616	5.552	5.584 ± 0.032
	0.0125	5.216	5.248	5.232 ± 0.016
	0.025	4.336	4.432	4.384 ± 0.048
	0.05	3.216	3.248	3.232 ± 0.016
2 Hours	Control	5.520	5.488	5.504 ± 0.016
	0.0125	5.024	4.992	5.008 ± 0.016
	0.025	3.872	3.904	3.888 ± 0.016
	0.05	1.984	2.048	2.016 ± 0.032

TABLE – 21. ESTIMATION OF RNA AFTER THE TREATMENT OF *ALLIUM CEPA* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF ETHION

Time	Concentrations of pesticide (%)	Amount of RNA in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	2.532	2.520	2.526 ± 0.006
	0.0125	1.764	1.788	1.776 ± 0.012
	0.025	1.578	1.590	1.584 ± 0.006
	0.05	1.350	1.362	1.356 ± 0.006
2 Hours	Control	1.758	1.794	1.776 ± 0.018
	0.0125	1.704	1.728	1.716 ± 0.012
	0.025	1.110	1.098	1.104 ± 0.006
	0.05	0.924	0.912	0.918 ± 0.006

TABLE – 22. ESTIMATION OF RNA AFTER THE TREATMENT OF *ALLIUM SATIVUM* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF ETHION

Time	Concentrations of pesticide (%)	Amount of RNA in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	4.110	4.080	4.095 ± 0.015
	0.0125	3.975	4.005	3.990 ± 0.015
	0.025	3.645	3.705	3.675 ± 0.030
	0.05	3.375	3.435	3.405 ± 0.030
2 Hours	Control	3.735	3.765	3.750 ± 0.015
	0.0125	3.435	3.345	3.390 ± 0.045
	0.025	2.130	2.100	2.115 ± 0.015
	0.05	1.725	1.785	1.755 ± 0.030

TABLE – 23. ESTIMATION OF RNA AFTER THE TREATMENT OF *ALLIUM CEPA* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF MALATHION

Time	Concentrations of pesticide (%)	Amount of RNA in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	2.532	2.520	2.526 ± 0.006
	0.0125	1.596	1.608	1.602 ± 0.006
	0.025	1.212	1.200	1.206 ± 0.006
	0.05	0.942	0.918	0.930 ± 0.012
2 Hours	Control	1.758	1.794	1.776 ± 0.018
	0.0125	1.248	1.260	1.254 ± 0.006
	0.025	0.786	0.822	0.804 ± 0.018
	0.05	0.612	0.648	0.630 ± 0.018

TABLE – 24. ESTIMATION OF RNA AFTER THE TREATMENT OF *ALLIUM SATIVUM* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF MALATHION

Time	Concentrations of pesticide (%)	Amount of RNA in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	4.110	4.080	4.095 ± 0.015
	0.0125	3.855	3.795	3.825 ± 0.030
	0.025	2.730	2.820	2.775 ± 0.045
	0.05	1.995	2.085	2.040 ± 0.045
2 Hours	Control	3.735	3.765	3.750 ± 0.015
	0.0125	3.315	3.375	3.345 ± 0.030
	0.025	2.325	2.385	2.355 ± 0.030
	0.05	1.965	1.995	1.980 ± 0.015

TABLE – 25. ESTIMATION OF RNA AFTER THE TREATMENT OF *ALLIUM CEPA* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF METACID

Time	Concentrations of pesticide (%)	Amount of RNA in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	2.532	2.520	2.526 ± 0.006
	0.0125	2.370	2.394	2.382 ± 0.012
	0.025	1.890	1.914	1.902 ± 0.012
	0.05	1.566	1.602	1.584 ± 0.018
2 Hours	Control	1.758	1.794	1.776 ± 0.018
	0.0125	1.722	1.686	1.704 ± 0.018
	0.025	1.518	1.530	1.524 ± 0.006
	0.05	0.882	0.894	0.888 ± 0.006

TABLE – 26. ESTIMATION OF RNA AFTER THE TREATMENT OF *ALLIUM SATIVUM* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF METACID

Time	Concentrations of pesticide (%)	Amount of RNA in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	4.110	4.080	4.095 ± 0.015
	0.0125	3.765	3.855	3.810 ± 0.045
	0.025	2.835	2.745	2.790 ± 0.045
	0.05	2.505	2.415	2.460 ± 0.045
2 Hours	Control	3.735	3.765	3.750 ± 0.015
	0.0125	3.465	3.585	3.525 ± 0.060
	0.025	3.180	3.270	3.225 ± 0.045
	0.05	2.520	2.460	2.490 ±0. 030

TABLE – 27. ESTIMATION OF RNA AFTER THE TREATMENT OF *ALLIUM CEPA* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF NUVAN

Time	Concentrations of pesticide (%)	Amount of RNA in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	2.532	2.520	2.526 ± 0.006
	0.0125	2.358	2.382	2.370 ± 0.012
	0.025	1.404	1.392	1.398 ± 0.006
	0.05	1.170	1.182	1.176 ± 0.006
2 Hours	Control	1.758	1.794	1.776 ± 0.018
	0.0125	1.380	1.368	1.374 ± 0.006
	0.025	1.122	1.134	1.128 ± 0.006
	0.05	0.768	0.732	0.750 ± 0.018

TABLE – 28. ESTIMATION OF RNA AFTER THE TREATMENT OF *ALLIUM SATIVUM* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF NUVAN

Time	Concentrations of pesticide (%)	Amount of RNA in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	4.110	4.080	4.095 ± 0.015
	0.0125	3.555	3.525	3.540 ± 0.015
	0.025	2.880	2.940	2.910 ± 0.030
	0.05	2.355	2.325	2.340 ± 0.015
2 Hours	Control	3.735	3.765	3.750 ± 0.015
	0.0125	3.300	3.420	3.360 ± 0.060
	0.025	2.715	2.835	2.775 ± 0.060
	0.05	2.175	2.235	2.205 ± 0.030

TABLE – 29. ESTIMATION OF RNA AFTER THE TREATMENT OF *ALLIUM CEPA* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF ROGOR

Time	Concentrations of pesticide (%)	Amount of RNA in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	2.532	2.520	2.526 ± 0.006
	0.0125	2.052	2.076	2.064 ± 0.012
	0.025	1.872	1.860	1.866 ± 0.006
	0.05	1.248	1.236	1.242 ± 0.006
2 Hours	Control	1.758	1.794	1.776 ± 0.018
	0.0125	1.662	1.674	1.668 ± 0.006
	0.025	1.098	1.134	1.116 ± 0.018
	0.05	0.774	0.762	0.768 ± 0.006

TABLE – 30. ESTIMATION OF RNA AFTER THE TREATMENT OF *ALLIUM SATIVUM* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF ROGOR

Time	Concentrations of pesticide (%)	Amount of RNA in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	4.110	4.080	4.095 ± 0.015
	0.0125	3.735	3.645	3.690 ± 0.045
	0.025	2.955	2.895	2.925 ± 0.030
	0.05	2.685	2.625	2.655 ± 0.030
2 Hours	Control	3.735	3.765	3.750 ± 0.015
	0.0125	3.255	3.165	3.210 ± 0.045
	0.025	3.015	2.955	2.985 ± 0.030
	0.05	2.265	2.325	2.295 ± 0.030

TABLE – 31. ESTIMATION OF BUFFER SOLUBLE PROTEIN AFTER THE TREATMENT OF *ALLIUM CEPA* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF ETHION

Time	Concentrations of pesticide (%)	Amount of buffer soluble protein in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	2.336	2.304	2.320 ± 0.016
	0.0125	1.496	1.464	1.480 ± 0.016
	0.025	1.336	1.352	1.344 ± 0.008
	0.05	1.072	1.104	1.088 ± 0.016
2 Hours	Control	2.088	2.072	2.080 ± 0.008
	0.0125	1.416	1.432	1.424 ± 0.008
	0.025	1.264	1.296	1.280 ± 0.016
	0.05	0.816	0.864	0.840 ± 0.024

TABLE – 32. ESTIMATION OF BUFFER SOLUBLE PROTEIN AFTER THE TREATMENT OF *ALLIUM SATIVUM* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF ETHION

Time	Concentrations of pesticide (%)	Amount of buffer soluble protein in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	3.112	3.144	3.128 ± 0.016
	0.0125	2.368	2.352	2.360 ± 0.008
	0.025	1.840	1.856	1.848 ± 0.008
	0.05	1.320	1.352	1.336 ± 0.016
2 Hours	Control	2.992	3.024	3.008 ± 0.016
	0.0125	1.952	1.936	1.944 ± 0.008
	0.025	1.784	1.816	1.800 ± 0.016
	0.05	1.248	1.264	1.256 ± 0.008

TABLE – 33. ESTIMATION OF BUFFER SOLUBLE PROTEIN AFTER THE TREATMENT OF *ALLIUM CEPA* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF MALATHION

Time	Concentrations of pesticide (%)	Amount of buffer soluble protein in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	2.336	2.304	2.320 ± 0.016
	0.0125	1.992	1.944	1.968 ± 0.024
	0.025	1.624	1.672	1.648 ± 0.024
	0.05	1.208	1.256	1.232 ± 0.024
2 Hours	Control	2.088	2.072	2.080 ± 0.008
	0.0125	1.792	1.808	1.800 ± 0.008
	0.025	1.560	1.576	1.568 ± 0.008
	0.05	1.024	0.976	1.000 ± 0.024

TABLE – 34. ESTIMATION OF BUFFER SOLUBLE PROTEIN AFTER THE TREATMENT OF *ALLIUM SATIVUM* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF MALATHION

Time	Concentrations of pesticide (%)	Amount of buffer soluble protein in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	3.112	3.144	3.128 ± 0.016
	0.0125	2.240	2.304	2.272 ± 0.032
	0.025	2.064	2.016	2.040 ± 0.024
	0.05	1.624	1.656	1.640 ± 0.016
2 Hours	Control	2.992	3.024	3.008 ± 0.016
	0.0125	2.192	2.176	2.184 ± 0.008
	0.025	1.888	1.856	1.872 ± 0.016
	0.05	1.576	1.592	1.584 ± 0.008

TABLE – 35. ESTIMATION OF BUFFER SOLUBLE PROTEIN AFTER THE TREATMENT OF *ALLIUM CEPA* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF METACID

Time	Concentrations of pesticide (%)	Amount of buffer soluble protein in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	2.336	2.304	2.320 ± 0.016
	0.0125	2.216	2.184	2.200 ± 0.016
	0.025	1.968	1.984	1.976 ± 0.008
	0.05	1.664	1.680	1.672 ± 0.008
2 Hours	Control	2.088	2.072	2.080 ± 0.008
	0.0125	2.248	2.280	2.264 ± 0.016
	0.025	1.824	1.776	1.800 ± 0.024
	0.05	1.640	1.672	1.656 ± 0.016

TABLE – 36. ESTIMATION OF BUFFER SOLUBLE PROTEIN AFTER THE TREATMENT OF *ALLIUM SATIVUM* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF METACID

Time	Concentrations of pesticide (%)	Amount of buffer soluble protein in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	3.112	3.144	3.128 ± 0.016
	0.0125	2.280	2.296	2.288 ± 0.008
	0.025	1.880	1.896	1.888 ± 0.008
	0.05	1.608	1.624	1.616 ± 0.008
2 Hours	Control	2.992	3.024	3.008 ± 0.016
	0.0125	2.320	2.384	2.352 ± 0.032
	0.025	2.184	2.216	2.200 ± 0.016
	0.05	1.544	1.576	1.560 ± 0.016

TABLE – 37. ESTIMATION OF BUFFER SOLUBLE PROTEIN AFTER THE TREATMENT OF *ALLIUM CEPA* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF NUVAN

Time	Concentrations of pesticide (%)	Amount of buffer soluble protein in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	2.336	2.304	2.320 ± 0.016
	0.0125	1.744	1.760	1.752 ± 0.008
	0.025	1.392	1.424	1.408 ± 0.016
	0.05	1.184	1.136	1.160 ± 0.024
2 Hours	Control	2.088	2.072	2.080 ± 0.008
	0.0125	1.496	1.512	1.504 ± 0.008
	0.025	1.184	1.168	1.176 ± 0.008
	0.05	0.912	0.944	0.928 ± 0.016

TABLE – 38. ESTIMATION OF BUFFER SOLUBLE PROTEIN AFTER THE TREATMENT OF *ALLIUM SATIVUM* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF NUVAN

Time	Concentrations of pesticide (%)	Amount of buffer soluble protein in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	3.112	3.144	3.128 ± 0.016
	0.0125	1.800	1.784	1.792 ± 0.008
	0.025	1.440	1.424	1.432 ± 0.008
	0.05	1.144	1.176	1.160 ± 0.016
2 Hours	Control	2.992	3.024	3.008 ± 0.016
	0.0125	1.608	1.656	1.632 ± 0.024
	0.025	1.320	1.336	1.328 ± 0.008
	0.05	1.080	1.064	1.072 ± 0.008

TABLE – 39. ESTIMATION OF BUFFER SOLUBLE PROTEIN AFTER THE TREATMENT OF *ALLIUM CEPA* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF ROGOR

Time	Concentrations of pesticide (%)	Amount of buffer soluble protein in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	2.336	2.304	2.320 ± 0.016
	0.0125	2.136	2.104	2.120 ± 0.016
	0.025	1.792	1.744	1.768 ± 0.024
	0.05	0.832	0.864	0.848 ± 0.016
2 Hours	Control	2.088	2.072	2.080 ± 0.008
	0.0125	1.456	1.504	1.480 ± 0.024
	0.025	1.168	1.184	1.176 ± 0.008
	0.05	0.864	0.816	0.840 ± 0.024

TABLE – 40. ESTIMATION OF BUFFER SOLUBLE PROTEIN AFTER THE TREATMENT OF *ALLIUM SATIVUM* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF ROGOR

Time	Concentrations of pesticide (%)	Amount of buffer soluble protein in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	3.112	3.144	3.128 ± 0.016
	0.0125	2.488	2.520	2.504 ± 0.016
	0.025	2.176	2.224	2.200 ± 0.024
	0.05	1.568	1.536	1.552 ± 0.016
2 Hours	Control	2.992	3.024	3.008 ± 0.016
	0.0125	1.816	1.784	1.800 ± 0.016
	0.025	1.672	1.688	1.680 ± 0.008
	0.05	1.424	1.360	1.392 ± 0.032

TABLE – 41. ESTIMATION OF TOTAL FREE AMINO ACIDS AFTER THE TREATMENT OF *ALLIUM CEPA* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF ETHION

Time	Concentrations of pesticide (%)	Amount of amino acids in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	1.530	1.680	1.605 ± 0.075
	0.0125	1.135	1.185	1.160 ± 0.025
	0.025	0.938	1.038	0.988 ± 0.050
	0.05	0.865	0.715	0.790 ± 0.075
2 Hours	Control	1.407	1.457	1.432 ± 0.025
	0.0125	0.962	1.112	1.037 ± 0.075
	0.025	0.864	0.964	0.914 ± 0.050
	0.05	0.714	0.814	0.764 ± 0.050

TABLE – 42. ESTIMATION OF TOTAL FREE AMINO ACIDS AFTER THE TREATMENT OF *ALLIUM SATIVUM* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF ETHION

Time	Concentrations of pesticide (%)	Amount of amino acids in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	2.272	2.246	2.259 ± 0.013
	0.0125	1.975	2.075	2.025 ± 0.050
	0.025	1.628	1.654	1.641 ± 0.013
	0.05	1.061	1.087	1.074 ± 0.013
2 Hours	Control	1.679	1.705	1.692 ± 0.013
	0.0125	1.581	1.555	1.568 ± 0.013
	0.025	1.013	0.987	1.000 ± 0.013
	0.05	0.666	0.716	0.691 ± 0.025

TABLE – 43. ESTIMATION OF TOTAL FREE AMINO ACIDS AFTER THE TREATMENT OF *ALLIUM CEPA* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF MALATHION

Time	Concentrations of pesticide (%)	Amount of amino acids in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	1.530	1.680	1.605 ± 0.075
	0.0125	1.184	1.434	1.309 ± 0.125
	0.025	1.036	1.136	1.086 ± 0.050
	0.05	0.740	0.890	0.815 ± 0.075
2 Hours	Control	1.407	1.457	1.432 ± 0.025
	0.0125	1.111	1.061	1.086 ± 0.025
	0.025	0.887	1.187	1.037 ± 0.150
	0.05	0.641	0.691	0.666 ± 0.075

TABLE – 44. ESTIMATION OF TOTAL FREE AMINO ACIDS AFTER THE TREATMENT OF *ALLIUM SATIVUM* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF MALATHION

Time	Concentrations of pesticide (%)	Amount of amino acids in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	2.272	2.246	2.259 ± 0.013
	0.0125	1.840	1.814	1.827 ± 0.013
	0.025	1.246	1.272	1.259 ± 0.013
	0.05	1.259	1.185	1.222 ± 0.037
2 Hours	Control	1.679	1.705	1.692 ± 0.013
	0.0125	1.136	1.062	1.099 ± 0.037
	0.025	0.950	0.900	0.925 ± 0.025
	0.05	0.827	0.777	0.802 ± 0.025

TABLE – 45. ESTIMATION OF TOTAL FREE AMINO ACIDS AFTER THE TREATMENT OF *ALLIUM CEPA* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF METACID

Time	Concentrations of pesticide (%)	Amount of amino acids in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	1.530	1.680	1.605 ± 0.075
	0.0125	1.951	1.801	1.876 ± 0.075
	0.025	1.382	1.432	1.407 ± 0.025
	0.05	0.864	0.914	0.889 ± 0.025
2 Hours	Control	1.407	1.457	1.432 ± 0.025
	0.0125	1.039	0.789	0.914 ± 0.125
	0.025	0.814	0.914	0.864 ± 0.050
	0.05	0.492	0.692	0.592 ± 0.100

TABLE – 46. ESTIMATION OF TOTAL FREE AMINO ACIDS AFTER THE TREATMENT OF *ALLIUM SATIVUM* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF METACID

Time	Concentrations of pesticide (%)	Amount of amino acids in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	2.272	2.246	2.259 ± 0.013
	0.0125	2.173	2.147	2.160 ± 0.013
	0.025	2.322	2.296	2.309 ± 0.013
	0.05	0.899	0.977	0.938 ± 0.039
2 Hours	Control	1.679	1.705	1.692 ± 0.013
	0.0125	1.283	1.309	1.296 ± 0.013
	0.025	1.197	1.223	1.210 ± 0.013
	0.05	0.854	0.776	0.815 ± 0.039

TABLE – 47. ESTIMATION OF TOTAL FREE AMINO ACIDS AFTER THE TREATMENT OF *ALLIUM CEPA* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF NUVAN

Time	Concentrations of pesticide (%)	Amount of amino acids in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	1.530	1.680	1.605 ± 0.075
	0.0125	1.358	1.408	1.383 ± 0.025
	0.025	0.986	1.236	1.111 ± 0.125
	0.05	0.864	0.814	0.839 ± 0.025
2 Hours	Control	1.407	1.457	1.432 ± 0.025
	0.0125	1.433	1.333	1.383 ± 0.050
	0.025	1.109	1.459	1.284 ± 0.175
	0.05	0.691	0.791	0.741 ± 0.050

TABLE – 48. ESTIMATION OF TOTAL FREE AMINO ACIDS AFTER THE TREATMENT OF *ALLIUM SATIVUM* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF NUVAN

Time	Concentrations of pesticide (%)	Amount of amino acids in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	2.272	2.246	2.259 ± 0.013
	0.0125	1.407	1.333	1.370 ± 0.037
	0.025	1.136	1.210	1.173 ± 0.037
	0.05	0.926	0.876	0.901 ± 0.025
2 Hours	Control	1.679	1.705	1.692 ± 0.013
	0.0125	0.987	1.037	1.012 ± 0.025
	0.025	0.963	0.913	0.938 ± 0.025
	0.05	0.765	0.839	0.802 ± 0.037

TABLE – 49. ESTIMATION OF TOTAL FREE AMINO ACIDS AFTER THE TREATMENT OF *ALLIUM CEPA* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF ROGOR

Time	Concentrations of pesticide (%)	Amount of amino acids in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	1.530	1.680	1.605 ± 0.075
	0.0125	0.937	1.137	1.037 ± 0.100
	0.025	0.764	0.964	0.864 ± 0.100
	0.05	0.666	0.716	0.691 ± 0.025
2 Hours	Control	1.407	1.457	1.432 ± 0.025
	0.0125	1.038	0.888	0.963 ± 0.075
	0.025	0.715	0.815	0.765 ± 0.050
	0.05	0.643	0.543	0.593 ± 0.050

TABLE – 50. ESTIMATION OF TOTAL FREE AMINO ACIDS AFTER THE TREATMENT OF *ALLIUM SATIVUM* ROOT TIP CELLS WITH DIFFERENT CONCENTRATIONS OF ROGOR

Time	Concentrations of pesticide (%)	Amount of amino acids in mg/gram fresh weight		
		1	2	Mean ± SD.
1 Hour	Control	2.272	2.246	2.259 ± 0.013
	0.0125	1.888	1.938	1.913 ± 0.025
	0.025	1.814	1.740	1.777 ± 0.037
	0.05	1.716	1.642	1.679 ± 0.037
2 Hours	Control	1.679	1.705	1.692 ± 0.013
	0.0125	1.492	1.692	1.592 ± 0.100
	0.025	1.395	1.445	1.420 ± 0.025
	0.05	1.123	1.197	1.160 ± 0.037

DISCUSSION

Kochuthressia M. V. "Cytotoxic and biochemical effects of some organophosphorus pesticides in Allium cepa L. and Allium sativum L." Thesis. Department of Botany, University of Calicut, 2005

DISCUSSION

A. CYTOTOXIC EFFECTS

Allium test has been used widely in scanning for the clastogenic effects of different chemicals, due to its relative simplicity (Kihlman, 1966). Grant (1978) pointed out that plant chromosomes are sensitive indicators to environmental pollutants and rightly suggested that the higher plant systems appear to be an excellent indicator of the cytotoxic, cytogenetic and mutagenic effects of environmental mutagens. Therefore the plant system is generally accepted as the first tier assay system for detection of the possible genetic damage resulting from the use of environmental chemicals. The present study revealed that, treatment of *Allium cepa* and *Allium sativum* root meristems with some organophosphorus pesticides had a detrimental effect on the test materials. Treatments not only brought down the frequency of dividing cells, but also produced a good number of anomalies in the mitotic cells and produced drastic changes in the amount of biochemical molecules. There were a marked decrease in the mitotic index and gradual increase in the percentage of chromosomal abnormalities as the concentrations of the experimental solution and the times of treatment increased. Such an inverse relation between the mitotic index and the dosage and time of treatment and a direct relation between the concentration and time of treatment to percentage of abnormalities had been reported earlier by various other workers working with different pesticides on various plants.

Cytological observations revealed that the five applied organophosphorus pesticides such as Ethion, Malathion, Metacid, Nuvan and Rogor used in the present study are capable of producing various mitotic abnormalities as well as biochemical changes. The induction of cytological disturbances in the treated mitotic cells is of great value, as it results in genetic damages that are handed over to the next generation. The frequency and types of aberrations recorded in the present investigations clearly indicate that the chemicals used here are

capable of inducing toxic effects on chromosomes of *Allium cepa* and *Allium sativum.* The results also show that there exists a relationship between the concentration and the percentage of aberration. The gross abnormalities are similar to the results obtained by Wuu and Grant (1967b) with Atrazine and Reddy and Rao (1981) with BHC and Nuvacron.

The cytotoxic chemicals act on mitotic cells in three different manners (Ray and Barman, 1987).

1. Pre-prophase inhibitor.
2. Inhibitor of mitotic spindle formation and orientation, compounds being termed as mitoclastic agents.
3. Inhibitor of cell plate and cell wall formation between daughter nuclei resulting in binucleate and multinucleate cells.

Different kinds of aberrations were classified into clastogenic aberrations attributable to the direct action in chromosomes and nonclastogenic or physiologic aberrations attributable to spindle abnormalities.

The effect of the different treatments of pesticides on mitotic activity expressed as mitotic index (MI), types and number of chromosomal abnormalities, the amount of DNA, RNA, buffer soluble protein and total free amino acids are shown in the tables 1-50. Mitotic activity was progressively inhibited as the concentration of the pesticides increased and the period of treatment were prolonged. The reduction in mitotic activity was accompanied by a depressive action on the amount of DNA, RNA, buffer soluble protein and total free amino acids. Increased concentration and/or prolonged period of treatment results in increased reduction in their amounts. This is particularly evident from a comparison of the values of these parameters in treated roots of *A. cepa* with their values in control. In general the reduction in mitotic activity and the amount

of DNA, RNA, buffer soluble proteins and total free amino acids in the roots of *Allium cepa* was more pronounced than in *Allium sativum.*

Antimitotic effects: -

Antimitotic effects of the pesticides under the present investigation manifested as mitotic inhibition, mitotic arrest at different stages and as various spindle abnormalities varied with pesticides, their concentrations, types of treatment and the test materials used (Figs.117-124). This mitotic depression may be due to the inhibitory effect of chemicals leading to decrease in mitotic index (Figs.117-120), which can result from obstruction of the onset of prophase or from an arrest of the subsequent mitotic phases. The chemicals probably owing to its action on the spindle function caused an arrest in metaphase, producing a high percentage of metaphase abnormalities. The reduction in mitotic index may be mainly due to the inhibitory action of the chemicals on the onset of mitosis inspite of metaphase arrest. This leads to the conclusion that the pesticides caused a partial effect on spindle formation and also showed a mitotic depressive effect. The pattern of mitotic inhibition induced by the five organophosphorus pesticides was more or less the same. However, in the case of Ethion, the lower dose treatments showed comparatively lesser mitotic inhibition than other pesticides during one-hour treatment (Fig.117).

Mitodepression is to be related to the phosphorylating and alkylating properties of chemicals (Rosenkranz and Rosenkranz, 1972; Dedek *et al.*, 1976). The most significant effect of the pesticides evidenced from the present observations was the arrest of the mitotic cycle either at interphase or at different stages in the M-phase in treated cells. Reduction in the number of dividing cells resulted either from mitotic inhibition or from mitotic arrest at interphase. Arrest at mitosis was a common occurrence among the treated materials.

Mitotic index (MI) is that fraction of the total number of cells scored in a meristematic tissue, which are actively engaged in mitosis, from prophase to telophase (Reiger *et al.*, 1976). In other words, it is the percentage of cells in M-phase out of the total number of meristematic cells. According to Mazia (1961), the inhibition of mitosis accomplished naturally or artificially by 1. Diverting cells from the preparations for divisions. 2. Inhibiting any one of the preparations for division. 3. Arresting cells at the transition stage between interphase and division. 4.Disabling the mitotic apparatus.

In all the treatments, the decrease in mitotic index value in the root meristems of *Allium cepa* and *Allium sativum* with increasing concentration is attributed to mitotic inhibition. A decrease in mitotic activity was clearly observed when the roots were treated with high concentrations. Such a decrease in the mitotic index indicates that pesticides interfere in the normal sequence of mitosis, thus preventing a number of cells entering the prophase. Such reduction in the mitotic activity could be due to the inhibition of DNA synthesis (Schneiderman *et al.*, 1971). Bell *et al.* (1976) also showed that exposing the root tips of *Vicia faba* to high concentrations of the herbicide (paraquet), led to inhibition of DNA synthesis. Most of the phenolic herbicides inhibit oxidative phosphorylation (Gaur and Beevers, 1959; Kandler, 1960). This may suggest that the organophosphorus pesticides such as Ethion, Malathion, Metacid, Nuvan and Rogor could have the same effect. The reduction of mitotic activity seem to be a common effect of most herbicides tested for their action on mitosis (Amer and Farah, 1983). Mitotic inhibition by herbicide has been attributed to blocking of mitotic cycle during interphase, which may result from prolonged G2 period, or to the inhibition of DNA synthesis (Chand and Roy, 1981). In the present investigation, the mitotic inhibitory effects exhibited by the above mentioned

five organophosphorus pesticides during the treatments might be due to the presence of mito-inhibitory principles in them.

The cell cycle can be inhibited and blocked in different ways by a variety of drugs at different stages- the G_1, S-phase, G_2, chromosome condensation stage, nuclear membrane breakdown, spindle assembly, micro tubule- kinetochore interaction, visible doubling of chromosomes, anaphase movement, spindle disassembling or cleavage (Taylor, 1973). The effect of antimitotic substances is said to be mitodepressive if mitotic index is decreased in the cell population studied or mitostatic if it results in the disappearance of all mitosis (Deysson, 1968). Thus in the present investigation, all the five organophosphorus pesticides seem to be mitodepressive. The mitodepressive activity of all the five pesticides are dose and duration dependent, with the exception of a very few treatments (Figs.117-120). All the chromatoclastic agents were at the same time very potent mitodepressive agents and this inhibition can persist for a long time even after the end of the treatment. It is obvious that every inhibition of DNA synthesis or every strong alteration of existing DNA can induce according to the intensity of action, either chromosomal disturbances or a mitodepressive effect.

The inhibition of cell division has been attributed to chemical changes brought about by mutagens in the cells (Lea, 1962) or radiation inactivation of the oxidative enzymes, which slow down cell division (Rubin and Methinsky, 1958).

Antimitotic substances have been used with some success in cancer chemotherapy. The word antimitotic substance include every substance that lessens the number of mitosis in a cell population whatever the mechanism of this reduction may be. The inhibitory effect theoretically can result from 1. Obstruction of the onset of prophase. 2. The arrest of one of the mitotic (M-phase) stage. 3. Inhibition of the mitotic apparatus. 4. Faulty separation of the

daughter chromosomes. In fact it has been observed that some mitotic stages are especially sensitive to inhibiting chemicals while some others have never been disturbed by substances so far known (Deysson, 1968).

Simon (1987) reported a correlation between mitodepression and percentage of cells with heteropycnotic nuclei in studies with EMS, DES and DMS and stated that mitodepression and chemotoxicity were related to the phosphorylating and alkylating properties of test compounds and attributed the decrease in mitotic activity to change in viscosity of cytoplasm brought about by the non-formation of the mitotic apparatus or due to gross chromosomal aberrations. In the case of alkylating agents, the hydrolytic products may bring about a change in the pH of the cells which affects the biosynthetic pathways or the hydrolytic products may act as mitodepressors. It is assumed that the functional integrity of DNA is essential for survival and damage to the genome may lead to cell death. It is also assumed that DNA damage normally initiates mitotic delay and repair can take place during the recovery period. Residual lesions, which are not repaired during recovery period, lead to chromosomal aberrations at mitosis (Scott and Zampetti-Bosseler, 1980).

The effect of pesticides on the chromosomal anomalies induced and on mitotic index can be correlated with the concentration of the chemicals as observed by other workers on *Allium* and other plant species (Levan and Tjio, 1948). The delay in the appearance of interphase nuclei in mitosis and changes in duration of the mitotic cycle in plants have been noted by Vant' Hoff and Wilson (1962) and Takatori (1965). Mitotic inhibitions in root meristem of *Allium fistulosum* have been demonstrated after the treatment with GA (Kato, 1955) and in *Allium cepa*, with kinetin, IAA and maleic hydrazide (Mc Manus, 1960). In *Vicia faba*, however, low concentration of GA enhances where as high concentration inhibits cell division (Prasad and Das, 1977).

Mitodepression seemed to be related to the inhibition of oxidative phosphorylation resulting in the inhibition of cell division (Kihlman, 1966). This is because, the development of the mitotic processes required energy. Most of the energy required by the cell is provided by oxidative phophorylation in mitochondria in the form of ATP. Amer and Ali (1969) also had observed the decrease in division frequency.

Jain and Sarbhoy (1988) used some chlorinated pesticides and reported partial or entire inactivation of spindle mechanism followed by scattering of chromosomes. He proposed that the cell division is an energy dependent process and there by the movement of chromosomes mainly depends upon the energy-generated system. The pesticide treatment disturbs the respiratory pathways resulting in low accumulation of energy containing and other essential compounds that is ATP, sugar and protein molecules etc. Alkylating agents are known to cause chromosomal breakage by binding to DNA regions rich in GC pairs.

The chromosome anatomy theory (Frey-Wyssling, 1938) seems partially responsible for chromosome movement, as the chromosomes possess energy in the form of adenosine triphosphate (ATP) necessary for their movement. The movement of chromosome is under the influence of ATP (Hoffman-Berling, 1954). Since ATP, sugar and protein metabolic pathways are interrelated, the alteration in sugar and protein synthesis may also affect chromosome movement. Amato (1949) observed recovery tendency of chromosomes after the addition of sugars like m-inositol. Cummins *et al.* (1966) reported that the protein which determine the duration of transition from metaphase to onwards are concerned with the transformation of chemical energy into the mechanical work of mitosis. This assumption is also strengthened by the fact that agents, which suppress the energy generating processes like glycolysis, respiration and oxidative

phosphorylation, inhibit cell division. From this information it is assumed that the used organophosphorus pesticides have the capacity to suppress the energy generating processes. Bullought (1952) opinioned that energy required is produced during a particular stage of the mitotic cycle. It has not been established that when mitosis begins, but it onsets when prophase ends. According to Swann (1954), energy is steadily released by respiration and stored in the form of some organic compounds and is utilized in starting the cell division. Epel (1963) reported that the rate of mitosis was closely related to the resultant level of ATP and mitosis could be blocked at any stage at appropriate time by adding optimum concentration of respiratory inhibitors. The oxygen deficiency is known to cause delay in the onset of division by arresting cells at metaphase (Gimenz and Lopez-Saez 1961). Amoore (1961a, b) reported that the cell division in pea roots depends upon the presence of oxygen and can be arrested by the lack of oxygen.

It is clear from tables (Table 1-10) that effects of these pesticides in reducing cell divisions were pronounced by increasing concentration, this may indicate that the pesticides interferes with the normal sequences of cell cycle to reduce the number of cell starting to divide at interphase. It is also possible to suggest that the reduction in mitotic activity was due to the inhibition of the DNA synthesis, which is considered as one of the major pre-requisites for a cell to divide. In this connection, Heiner (1971) found that diethyl sulphate caused a blockage of DNA synthesis, which in turn induced a substantial mitotic delay. Davids (1973) as well as Swietlinska *et al.* (1974) reported that the mitotic inhibition was accompanied by DNA synthesis inhibition.

Mitotic inhibition by herbicides has been attributed to blocking of mitotic cycle during interphase, which may result from a prolonged G_2 period or to the inhibition of DNA synthesis (Scott 1968). The results of the present study

pointed out a close correlation between the action of pesticides (Ethion, Malathion, Metacid, Nuvan and Rogor) on mitosis and DNA, which is compatible with the hypothesis that inhibition of mitosis may be due to an inhibitory effect by the pesticides on DNA. Vant' Hoff (1968) suggested that the inhibition of mitotic activity by chemical compounds is due to an increase in the G_2 period. Webster and Davidson (1969) and Mac Leode (1969) attributed the inhibition of mitosis to the increase in S-phase duration.

A successful completion of mitosis is a result of integrated harmonious function of the cell as a whole. Presence of pesticide molecules or their derivatives may disturb the synthesis or the structure of DNA (Mitchison, 1977) or may cause inhibition of cells entry into mitosis (Kihlman, 1966) either by delay in the onset of G_2 (Gelfant, 1963) and/or S-phases (Mitchison and Creanor, 1971). Arrest of division would take place in most of these eventualities.

Results obtained during the present study revealed both the clastogenicity and nonclastogenicity of the five organophosphorus pesticides, which is evident from the direct actions on the chromosomes and the manifestation of spindle abnormalities. The capacity of a toxin to induce clastogenic aberrations may be regarded as an indication of its genotoxic potential (Hanaa *et al.*, 2000). The reduction of mitotic indices might have been achieved by the inhibition of DNA synthesis at S-phase (Sudhakar *et al.*, 2001). It may be due to the slowing of the rate of cell progression through mitosis or due to the obstruction of the onset of prophase or due to the arrest of mitotic phases (Kabarity and Mallalah, 1980). Shehab (1985) reported that chemical principles are capable of causing cytotoxic effect. The essential oil obtained from several plants has been shown to exhibit mitodepressive effects on the division stages of *Allium cepa, Allium sativum* and *Vicia faba* (Khandelwal, 1986; Wilson *et al.*, 1997 and Foray *et al.*, 1999).

On the basis of the mitotic indices, the antimitotic effects of the above five organophosphorus pesticides are compared and ranked in *Allium cepa* and *Allium sativum.* In *Allium cepa* Rogor was found to be the most effective antimitotic pesticide followed by Nuvan, Metacid, Malathion and Ethion. In *Allium sativum* on the other hand, Rogor caused severe mitotic depression followed by Metacid, Nuvan, Ethion and Malathion. In the present set of experiments, the lowering of mitotic indices in different treatments with the pesticides against the respective control evidenced mitotic inhibition. Hence it may be concluded that the cell division is an energy dependent process and there by the movement of chromosomes mainly depends upon the energy generating system. The pesticides treatment somehow disturbed the respiratory pathways, resulting in the low accumulation of energy containing molecules and other essential compounds – ATP, sugar and protein molecules etc. The present study reveals that the used organophosphorus pesticides such as Ethion, Malathion, Metacid, Nuvan and Rogor caused more hindrance in chromosome movement than other screened effects under the same condition. The disturbance in chromosome movement may be due to the combined action of other isomers also. Hence it seems that more than one factor in combination may be responsible for chromosome movement.

The various treatments of pesticides induced a variety of chromosomal abnormalities. In the present work, treatment with Ethion, Malathion, Metacid, Nuvan and Rogor produced an increased number of abnormal mitotic cells as compared to control material. There was a gradual increase in the percentage of chromosomal aberrations when the pesticide concentration was increased and the period of treatment prolonged. These results are in line with the results of many scientists who worked with different chemicals on different plant materials (Al-Najjar and Soliman, 1980; Rabindra *et al.* 1989).

The mitotic inhibitory effects are shown in the tables and figures. (Table 1-10, Figs.117-120). The percentage of abnormalities is generally increased as the concentration increased and the period of treatment was prolonged (Figs.121-124).

The pesticide induced chromosomal abnormalities can be broadly put into two categories. Different kinds of aberrations were classified into clastogenic aberrations attributable to the direct action in chromosomes and nonclastogenic or physiologic aberrations attributable to spindle abnormalities.

The frequency and types of aberrations recorded in the present investigation clearly indicate that the chemicals used are capable of inducing toxic effects on chromosomes of *Allium cepa* and *Allium sativum*. *A. cepa* is more sensitive to the organophosphorus pesticides used than *A. sativum*. The induction of cytological disturbances in treated mitotic cells is of great value, as it results in genetic damage that is handed over to the next generation. The results also show that there exists a relationship between the concentration of the pesticide and the percentage of aberration. The gross abnormalities are similar to the results obtained by Amer and Farah (1976) with Sevin, Rogor and Duphar; Reddy and Rao (1968) with Demecrona and Rogor and Venkatarajan (1981) with EMS. The wide spread use of an increasing number of chemicals in agriculture and the resulting contamination of food with an increasing variety of pesticide residues imposes several problems. Apart from the dangers of acute and chronic poisoning, the possible mutagenic effects of such contamination could threaten the genetic health of coming generation and it is therefore of high importance. The cytotoxic effects of all the five pesticides studied include many clastogenic and non-clastogenic abnormalities.

The cells, which enter division from interphase after exposure to the treatment, show structural changes and the cells, which are already in division at

the time of treatment, show physiological effects. The mitotic poison may interfere with the synthesis, state and structure of nucleic acid including physiological effects and structural changes in chromosomes during cell division, which may lead to mitotic delay and mitotic inhibition. The changes brought about by the viscosity of the cytoplasm are primarily responsible for chromosome abnormalities like unequal separation, formation of fragments during anaphase, polyploidy, chromatid separation, micronuclei formation, multinucleate cell etc. (Sharma, 1980).

A comparison of the clastogenic and non-clastogenic effects caused by the applied five organophosphorus pesticides of the present study showed wide variation among themselves. In all the experiments, the predominant type of cytotoxic anomalies noticed came under the non-clastogenic category. Earlier researchers have an opinion that the chemicals present in the pesticides might have a direct action on the chromosome, DNA, RNA, and protein synthesis. The results were manifested both at the nuclear and chromosomal levels. Sometimes these chemicals might have been responsible for the changes in the viscosity of the cytoplasm also. The physiological or non-clastogenic effects might have been caused due to spindle abnormalities or inhibition in spindle formation. The major physiological and clastogenic abnormalities recorded in the present investigation are given below:

Misorientation of chromosomes: - Misorientation of chromosomes observed (Tables 2, 6, 7, 8, 9; Figs. 21, 44, 63, 84, 102) during this study may be due to the disturbed functioning of the spindle apparatus. The disturbance can be due to a distortion of the spindle apparatus, a tilt in the equatorial organization of metaphase chromosomes or a change in the direction of movement of daughter chromosomes during anaphase (Saliem *et al.*, 1981).

The hindrance of the movement of the bivalents to the equatorial plate usually results in non-orientation of chromosomes. Such phenomenon was also observed in *Vicia faba* by treating with some phenols and herbicides (Amer and Ali, 1968).

Highly irregular chromosomes at metaphase: - Highly irregular chromosomes at metaphases were noticed only after the treatment of *Allium cepa* root tip cells with the pesticide Nuvan (Table 7; Fig. 22). In this case, chromosomes stick and spread irregularly over the cell. This may be due to the interference of pesticide Nuvan on the spindle apparatus.

Dislocation of chromosomes: - Dislocation of chromosomes at metaphase was also found in the treatment of *Allium cepa* with Ethion, Malathion Metacid, Nuvan, Rogor and *A. sativum* with Metacid (Tables 1, 3, 5, 6, 7, 9; Figs. 24, 28, 45). The chemicals present in the pesticide Malathion may affect the spindle apparatus. So dislocation may be attributed to the failure of the spindle apparatus to organize and function in a normal way.

Stickiness of chromosomes: - Stickiness is the major type of chromosomal abnormality recorded in the root tips of treated population (Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, 10; Figs. 23, 25, 27, 28, 36, 37, 40, 42, 43, 54, 57, 58, 63, 64, 65, 66, 67, 77, 79, 81, 82, 101, 112). This confirms the assumption that stickiness is a common physiological phenomenon induced in the root tips after the pesticide treatment. This seems to be a temporary effect since the percentage of stickiness is very high in metaphase when compared to anaphase and telophase. Darlington (1942) reported that stickiness is due to the disturbances in the nucleic acid metabolism of the cell. It may be due to the degradation or depolymerization of chromosomal DNA (Darlington and Mc Leish, 1951). It has been attributed to improper folding of chromosomal fibers, that makes chromatids connected by means of subchromatid bridges (Badr and Ibrahim, 1987). Stickiness has also

been attributed to the reaction of the applied pesticides with DNA, protein or both of them forming inter and intra chromosomal cross- links (Patil and Bhat, 1992). Induction of these abnormalities indicates the magnetic potential of the applied pesticides. Stickiness has been interpreted to be the result of partial dissolution of nucleoproteins (Kaufman, 1956), breakage and exchange of the basic folded fiber unit of chromatids (Klasterska *et al.* 1976). Stickiness is a type of physical adhesion involving mainly the proteinaceous matrix of the chromatin material (Stephen, 1979). Stickiness and condensation of chromosomes below their normal size might also be due to delay in chromosome movement after treatment with pesticides. The size of the chromosomes in different stages of the cell cycle is controlled by genes/gene. As a result of pesticide treatment, the chromosomes could not reach to the poles and remain scattered in cytoplasm. So due to gene action, the chromosomes look condensed and sticky in appearance.

Chromosome stickiness may be due to the action of the pesticides on the protein (El-Sadek, 1972), which form an integral part of the chromosomes. The sticky nature of chromosomes is probably due to the heterochromatinization of chromosomes resulting in the denaturation of nucleic acid and thus making the chromosome content adhesive (Grundman, 1966). Induction of stickiness of chromosomes is attributed to the cytotoxic effect of the chemical substances (Panda and Sahu, 1985).

Stickiness is the chromatid type of aberration (Klasterska *et al.*, 1976). It may result from the entanglement of chromatin fibers, which fail to condense properly in preparation for mitosis (Mc Gill *et al.*, 1974). There could be some substance present in the pesticide, which affects the DNA structure perhaps resulting in physical depolymerization of DNA. This together with or without partial dissolution of nucleoproteins could account for the stickiness of chromosomes (Mercykutty and Stephen, 1980). Various degrees of stickiness

were observed starting from a tendency for stickiness among chromosomes to extreme cases of stickiness where the chromosomes were seen as a mass or masses of chromatin due to the amalgamation of chromosomes.

Diagonal orientation of chromosomes: - Diagonal orientation of chromosomes was a frequent abnormality found in the treated root tip cells of *Allium cepa* and *Allium sativum*. There were polarity changes in the spindle orientation and thus orientation of chromosomes became diagonal to the cell in metaphase, anaphase and telophase (Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, 10; Figs. 26, 27, 47, 49, 51, 54, 61, 80, 87, 91, 93, 94, 96 and 113). It is a physiological anomaly noticed in the treated root tip cells. This may be due to the improper functioning of spindle apparatus induced by chemicals present in the pesticides. According to Das *et al.* (1968) the improper functioning of spindle apparatus causes the diagonal orientation of chromosomes. Dawey and Miller (1969) and Vig (1971) also noticed this phenomenon with X-rays and colchicine.

C-metaphase: - Cell at C-metaphase is observed in treatments of *Allium cepa* with Malathion, Metacid and Nuvan (Tables 3, 5, 7; Fig.30). Treatments of *Allium satvium* with Ethion and Nuvan also exhibt C-metaphase (Tables 2, 8; Fig. 85). Levan (1938) termed the scattering of chromosomes by spindle inhibition as c-mitosis or colchicine mitosis. The spindle abnormalities lead to C-metaphase and at the same time even causes the breakage of metaphase chromosome (Nagl, 1970). Sharda *et al.* (1973) suggested that the c-mitosis is the secondary effect of a prolonged metaphase. The C-metaphase is produced as a result of inhibition of spindle fiber formation also. Deysson (1968) suggested that C-metaphase might lead to the formation of polyploid cells.

According to Redei (1998) in the event of c-mitosis, the poisonous effect of the cytotoxic chemical blocks the mitotic anaphase and consequently the cell and its progeny may become polyploid. The c-mitosis is one of the consequences

of inactivation of spindle apparatus connected with the delay in the division of centromere (Gomergen, 2000). The chromosomes were found scattered and chromatids become clearer in C-metaphase. The action of these pesticides like the action of colchicine may be explained by disturbance in the synthesis of proteins, nucleic acids and antagonism between these substances (Dawey and Miller, 1969). The analysis revealed that the pesticides are quite effective in producing aberrant cells even at very low doses.

The C-metaphase observed in the present study appears to be the cytological effects of the inhibition of protein synthesis during mitosis. It is also possible that the used pesticides' binds with the DNA, there by preventing it from unwinding for transcription of spindle protein messengers.

C-metaphase cells after restitution are known to give rise to polyploid cells. Arrested metaphase and polyploid cells indicated that, the pesticides in the present investigation caused inhibition of spindle formation similar to the effect of colchicines.

Clumping: - The pesticide treated cells in metaphase show clumping. Chromosome clumping was a more frequent abnormality found in the cells of *Allium cepa* and *Allium sativum* root meristem cells after treating with pesticides (Tables 1, 2, 4, 6, 7, 10; Figs. 31, 78). Clumping is complete in some cells and the clumped chromosomes appear as a stained mass. If chromosome separation takes place at this stage, it will result in the bridge formation. Clumping of chromosomes is attributed to the increased concentration of the cytotoxicant (Pritchard and Court, 1966). Hadder and Wilson (1958) considered the scattered and clumped metaphases as the partial and full effect respectively of a C-mitotic agent. It may be due to the action of pesticides on chromosome-to-chromosome spindles and pole-to-pole spindles. So the chromosome-to-chromosome distance has changed and were seen clumped together. The above mentioned causes seem

to be responsible for the occurrence of clumping in *Allium cepa* root meristem after treatment with Ethion and Nuvan (Tables 1, 7) and *Allium sativum* root tip cells with pesticides Ethion, Malathion, Metacid and Rogor (Tables 2, 4, 6, 10).

Star metaphase/anaphase: - Star metaphases/anaphases were observed in *Allium cepa* and *Allium sativum* root tip cells after treatment with the organophosphorus pesticides Metacid and Rogor (Tables 5, 6, 9; Figs. 29, 55, 90, 101). Such type of anomaly was also observed after treatment of *Allium cepa* roots with pure "Sevin"(Amer, 1965) and considered as being a fore-step of the complete disturbance of the spindle. Star shaped arrangement may be due to the clumping of daughter chromosomes into star like structures near the polar region of the cell. Ostergen *et al.* (1953) have described the occurrence of double star anaphase in higher plants. According to them, metaphase and early mid-anaphase were usually normal. The chromosome splits and are distributed at random. After sister chromatids reach the polar regions, they are finally arranged around two poles like two monasters with their arms pointing outward, giving rise to a double star anaphase. Phragmoplast formation is disturbed and the cell plate simply does not form, resulting in the formation of a binucleate cell. Bloom *et al.* (1955) and Zirkle (1970) also observed the formation of double star anaphase after UV micro beam irradiation.

Ball metaphase: - It was observed in the treatment of *Allium cepa* with Ethion and Nuvan and also *Allium sativum* with Malathion (Tables 1, 4, 7; Figs. 32, 76). Ball metaphase is followed by either a complete degeneration of the cell or a state similar to interphase (Barber and Callan, 1942). In the present study, it may be due to the localized activity of spindle apparatus at the centre, so that chromosomes were arranged in such a way that centromeres remain at the equator and arms radiating in different directions and orienting in the form of a ball.

Unequal segregation and association: - Unequal segregation and abnormal association of chromosomes were noticed in the pesticide treated cells of *Allium cepa* and *Allium sativum* (Tables 3, 8, 10; Figs. 33, 39, 98, 102). This was explained as the partial suppression of spindle action by disturbances in the synthesis of spindle protein.

Oblique metaphase: - Oblique metaphase was of common occurrence in Malathion treated cells of *Allium sativum* only (Table 4; Fig. 83). It is a physiological anomaly formed due to the malfunctioning of spindle apparatus induced by the chemicals found in the pesticide Malathion.

Scattering of chromosomes: - Scattering of chromosomes was noticed after the treatments of *Allium cepa* root tip cells with the pesticide Rogor (Table 9; Fig. 34). It could be attributed to the interference of pesticide with tubulin and/or polymerization of the microtubular subunits forming the spindle apparatus. Chromosomes spread irregularly over the cell due to the disturbance of the spindle apparatus. Such types were also reported in *Vicia faba* and *Gossypium barbadense* after seed soak and root treatment with Sevin (Amer *et al.* 1971). In the present study, scattering of chromosomes might be due to the above reason.

Polyploidy: - Polyploidy was of common occurrence in the course of investigation carried out to study the cytotoxicity of Metacid on root meristems of *Allium cepa* (Table 5). The chemicals present in the pesticides inhibit cell plate formation and induce polyploid cells or hyperploid cells (Figs. 68, 82). Cells containing multiples of normal chromosome number, make them less competitive than normal ones and thus only a few of the aberrations of this kind are likely to be transmitted to subsequent generations (Liang *et al.*, 1969). Herichova (1973) noticed polyploid cells in mitosis of barley after the application of spindle destructing chemicals. The occurrence of hyperploid cells may be attributed to the spindle inhibition, lack of anaphase movement or failure

of cell plate formation (Nagpal and Grover, 1994). According to Onfelt and Klasterska (1983), mitotic abnormalities are generally considered insignificant from the mutation point of view unless polyploid cells are induced.

During the present study, it has been observed that the most significant effect of Metacid is its capacity to inhibit cytokinesis. Under the turbogenic action of Metacid, normal spindle formation is inhibited. Formation of polyploidy in these treatments found to be related to the loss of normal spindle activity followed by inhibition of cell plate formation. Hence in the present investigation the turbogenic potential of the pesticide Metacid is revealed, which is significant from the mutation point of view.

Ring chromosome: - A small ring chromosome was noticed after the treatment of *Allium cepa* with Malathion, Metacid, Nuvan (Tables 3, 5, 7) and *Allium sativum* with Malathion and Metacid (Tables 4, 6; Figs. 38, 42, 52, 53, 67, 88). Induction of ring chromosomes suggests the possibility of two breaks that occur in the same chromosome. Such ring chromosomes have been observed following X-ray and gamma irradiation. Sax (1940) opinioned that the two breaks that occurs in the same chromosome, after the process of rejoining may form a ring chromosome. Raghuvanshi and Singh (1976) attributed the formation of ring chromosomes due to telomeric losses. The cytotoxic chemicals present in the pesticides act directly on the fragile sites of the chromosomes leading to breakage at the terminal region and reunion of the raw ends of the chromosome so as to form a ring.

Multipolarity: - Multipolar spindles observed during this study may be due to the disruption of the spindle poles (Tables 2, 3, 4, 8, 9; Figs. 39, 53, 97, 99, 100, 109, 111). A possible explanation might be that the inactivation of spindle apparatus was so much pronounced that the chromosomes could not regain the normal position inside the cell. Position and number of poles determine

multipolar condition. The number of poles in a cell depends on the position of the assemblage of RNA and polysaccharides, which remain, distributed either in the form of sol or gel. (Prasad, 1974). This abnormality seems to be specific and is produced by certain chemicals not by all. Multipolar spindles and other spindle abnormalities were reported in canary grass after irradiation (Prasad, 1974) and in *Pisum sativum* after EMS and MMS treatment (Narasinghani and Kumar, 1976), which were attributed to abnormal spindles. The organization of spindle poles is disrupted and they diffuse to many directions instead of two poles (Therman and Timomen, 1950). Multipolar mitosis resulted when spindle fibers were badly disrupted. They were produced after prolonged treatment of mammalian cells *in vitro* with benzamide (Babu *et al.*, 1980) and were reported to be due to abnormal behavior of centrioles. According to Bajar and Mole Bajar (1969), in the early stages of prometaphase, the spindle may be multipolar before it changes to bipolar. However, very rarely does this multipolar condition persists and results in a multipolar metaphase-anaphase (Bajar and Mole Bajar, 1972). Tripolar cells indicate the inhibition of cytokinesis (Somasekhar and Gouda, 1984). The error in spindle organization even leads to multipolar spindle (El-Khodary *et al.*, 1989a). In the present investigations the tripolar cells may be due to change in physiological milieu by these organophosphorus pesticides.

Stathmo-anaphase: - Stathmo-anaphase is another type of chromosomal abnormality found in the treatment of *Allium cepa* root meristem with Rogor (Table 9; Fig. 41) and *Allium sativum* root tip cells with Nuvan and Rogor (Tables 8, 10; Figs. 93, 104). Here the daughter chromosomes do not separate fully, but they remain connected together by means of partial overlapping of the arms. This may also be due to the abnormal functioning of spindle fibers. Since the applied pesticides are having toxic activities on the spindle, they can be considered as stathmo- kinetic agents (Shehab, 1979).

Early movement: - Early movement is observed in both *Allium cepa* and *Allium sativum* after the treatment with different pesticide concentrations (Tables 1, 2, 3, 5, 6, 8, 9, 10; Figs. 46, 47, 48, 53, 87, 91, 95, 103). The precocious movement of the chromosomes might have been caused by the early terminalisation, resulting in the movement of the chromosome ahead of the rest during anaphase (Kaur and Grover, 1985). The abnormalities manifested may be due to the action of organic and inorganic molecules present in the pesticides on the mitotic spindle apparatus leading to its malfunctioning.

Lagging chromosome: - Lagging, the retardation of chromosome movement and delayed terminalization forces the laggards to remain outside the daughter nuclei in telophase. Lagging chromosomes are not in keeping pace with anaphase movements. They were found in *Allium cepa* root tip cells when treated with Ethion, Metacid and Rogor (Tables 1, 5, 9; Figs. 52, 59) and in *Allium sativum* after treatment with Ethion, Malathion and Rogor (Tables 2, 4, 10; Figs. 86, 109, 114, 116). The phenomenon of lagging chromosomes may be attributed to hindrance of the pre-metaphase movement accompanied by adhesion of the centromeres to the nuclear membrane or to the surrounding surface of the plasma membrane. Adhesion of the centromeres of one or more chromosomes to the outer layer of the plasma and movement of the other towards the equatorial plate lead to the appearance of such lagging chromosomes (Barthelmess, 1957). Induction of laggards could be attributed to the failure of the spindle apparatus to organize and function in a normal way (Patil and Bhat, 1992).

According to Lazanyi (1968), lagging chromosomes or laggards show retarded movement or no movement at all compared to the rest of the chromosomes during mitotic or meiotic metaphase and anaphase. The lagging chromosomes failed to be included in one of the daughter nuclei leads to the formation of micronuclei. Lagging chromosomes result from abnormal

functioning of the spindle, and loss or inactivation of kinetochores. In certain instances, the lagging chromosomes appear to have less active kinetochores (Nesy, 1991). Lagging chromosomes may also arise as a result of centromere deletion or inactivation as well (Reider, 1982). Singh (1982) has expressed the view that the lagging of these chromosomes at anaphase might be due to the inactivation of chromosomal spindle fibers attached to them. Lagging of chromosomes and their subsequent elimination can well be attributed to the after effect of the centromeric inactivation (Chacko, 1981). Carlson (1938) induced such lagging in cells by some other agents including UV micro beam. Lagging is a consequence of centromere inactivation and is the result of partial mitoclastic events (Lazanyi, 1968).

The lagging of chromosomes observed in the present study also might be due to the disruption of the spindle fibers attached to the chromosome or due to the inactivation of the kinetochores. The chance for the occurrence of the above mentioned molecular effects or a combination of several effects seem to be the probable reason behind the occurrence of chromosome laggards in *Allium cepa* and *Allium sativum* root meristems in the present investigation. Such laggards may araise due to the disturbance of the spindle apparatus.

Disturbed pro-meta-ana-telophases: - The chromosomes spread irregularly all over the cell (Fig. 92) in the case of disturbed metaphase, anaphase and telophases. According to Kabarity (1962) in disturbed prophase, the arrangement of the chromatin thread was abnormal (Fig. 69). This abnormality was present in the treatment of *Allium sativum* with Malathion and Metacid (Tables 4, 6). Inhibition of spindle formation lead to disturbed meta-ana and telophases (Figs. 34, 59, 92). Many workers referred to the affectivity of certain pesticides in inducing spindle inhibition (Mousa, 1982; Amer and Mikhael, 1986). Formation of this abnormality indicates that the applied pesticides induced partial inhibition

of mitotic apparatus. The compounds that produce this action were regarded as mitotic poisons. This may be an indication that, most of the abnormal phases continued their mitotic cycle till the ana-telophase stages.

Jain and Sarbhoy (1988) reported partial or entire inactivation of spindle mechanism followed by scattering of chromosomes. He proposed that the cell division is an energy dependent process and there by the movement of chromosomes mainly depends upon the energy-generated system. The pesticide treatments disturb the respiratory pathways resulting in low accumulation of energy containing molecules and other essential compounds i.e., ATP, sugar and protein molecules.

Non-synchronous movement: - The applied pesticides Nuvan and Rogor caused non-synchronous movement of chromosomes in *Allium sativum* (Tables 8, 10; Figs. 102, 107). It may be due to the severe imbalances in the spindle mechanism. It may lead to the multipolar nature of the mitotic spindle apparatus caused by the major chemical principles of the pesticides Nuvan and Rogor.

Chromosome fragmentation: - Fragmentation of chromosomes observed in the present investigation might be due to the stretching of chromosomes at metaphase leading to the breakage of chromosomes at centromere or other parts of the chromosome (Tables 1, 2, 5; Figs. 23, 52, 79, 108). Similar results were also reported after treatment with ionizing radiations and chemicals (Sparrow, 1961). Insecticidal and pesticidal agents such as gamaxane and DDT are also known to produce chromosome breakage (Amato, 1950). Amer and Ali (1968) showed that pesticides and phenolic compounds induce breakages during meiosis and most of the breaks were found to be of the chromatid type. Chromatid breaks are known to result from effects exerted during late interphase or early prophase when the chromosomes have already duplicated. The presence of chromosome and chromatid breaks tends to show that these pesticides exert to influence both

at early and late interphase. It may be assumed that chemicals besides producing extensive chromosomal breaks may simultaneously interfere with the normal functioning of repair enzyme and series of action involved in the process of rejoining.

The fragments might be due to the stickiness of the chromosomes and the consequent failure of the separation of chromatids to poles. Bruhin and Warner (1954) also reported the similar results when they used insecticides 5-5-dimethyl dihydro-resorcin and dimethyl carbamate on *Vicia faba*. Systox also caused chromosome fragments in root tip cells of *Vicia faba* (Bhattacharya, 1955). Occurrence of fragments at metaphase may be attributed to the failure of broken chromosomes to recombine. A number of organophosphorus pesticides have been reported to be radiomimetic and induce chromosomal breaks (Amer 1965; Amer and Farah, 1974).

Chemically induced chromosome breaks are more localized. The chemicals can induce direct breakage on chromosomes. The disruption of H-bond is regarded as principally responsible for the induction of chromosome breaks.

In the present study fragmentation is an important chromosomal anomaly. The above-mentioned causes either alone or in combination may be responsible for chromosome fragmentation.

Erosion: - Chromosome erosion was seen in *Allium cepa* root tip cells treated with Nuvan and Rogor (Tables 7, 9; Figs. 34, 56) and *Allium sativum* treated with Malathion (Table 4), indicated that certain sites in the chromosome became devoid of nucleic acid supply and appeared as thinner regions when compared to other region of chromosomes.

Pulverization: - Pulverization of chromosomes was observed in the treatment with Rogor in *Allium cepa* only (Table 9; Fig. 35). This abnormality is assumed

to be due to the premature condensation of chromosomes (Sakari *et al.*, 1981). In the present study, chromosome pulverization may be due to the reason envisaged by earlier workers.

Chromosome Bridges: - Single, double or multiple bridge formation was a major abnormality observed in several experiments conducted with the organophosphorus pesticides such as Ethion, Malathion, Metacid, Nuvan and Rogor (Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, 10; Figs. 37, 40, 42, 43, 44, 46, 50, 51, 54, 60, 62, 65, 66, 67, 89, 95, 100, 103, 105, 106, 108, 110, 114 and 115). It is a clastogenic action of applied pesticides on the chromosomes. All the pesticide applied induced anaphase and telophase bridges. This may be due to the unequal exchanges or dicentric chromosomes. The occurrence of breaks at the same locus and their lateral fusion leads to the formation of dicentric chromosomes. The dicentric chromosomes are pulled equally to both the poles at anaphase and bridge is formed (Sax, 1940). Single, double and even multiple bridges were noticed in the anaphase of both test materials.

The chromosomal bridges may be the result of reunion of broken chromosome ends. However in the present study, the frequency of bridges is too high. The bridges scored here may be the result of chromosomal stickiness, which may cause the chromosomes to remain connected through bridges at ana-telophases. Jadhav and Mungikar (1998) and Patnaik *et al.* (1984) also reported bridge formation. The bridges may be formed due to the breakage and fusion of chromosomes (Kaur and Grover, 1985) or failure of terminalization.

In the present study, the presence of chromatid bridges were also observed, indicating that the chromosome as well as the chromatids may be a primary target of pesticides.

Interphase anomalies observed in the treated root tip cells of *Allium cepa* and *Allium sativum*: -

After the treatment with different concentrations of pesticides Ethion, Malathion, Metacid, Nuvan and Rogor, the root tips of *Allium cepa* exhibited nuclear lesions, elongated nuclei showing prominent lesions, sickle shaped nucleus, binucleate cells with lesions, dumbbell shaped nucleus, nuclear deformation and nuclear extrusion. Where as interphase aberrations observed in *Allium sativum* were disturbed prophase, nuclear lesion, micronucleus, binucleate cell, and binucleate cell with lesion, nuclear extrusion and nuclear deformation. On comparison it was found that the pesticides induced less number and type of aberrations in *Allium sativum*. However the interphase anomalies present in the treated root tips of *Allium sativum* were also observed in *Allium cepa*.

Nuclear lesions: - In the present study nuclear lesions were observed in both the test materials after the treatment of pesticides (Tables 2, 3, 5, 6, 7; Figs. 9, 10, 17, 70, 73). Mercykutty and Stephen (1980) noticed the occurrence of nuclear lesions induced by plant-derived chemicals in *Allium cepa* root meristematic cells which may be due to the disintegration of portion of nuclear material by the action of the plant extracts.

In the light of the above sighted reference it seems probable that the occurrence of nuclear lesions may be due to the disintegration of the chromatin content resulting from the action of the toxic chemical principles present in the pesticides.

Micronucleus: - Interphase cells with micronucleus were noticed in root tip cells of *Allium cepa* and *Allium sativum* (Tables 1, 4, 6, 7, 10; Figs. 12, 71). Interphase cells with micronuclei were also observed in the mitosis of *Vicia faba* as a result of treatment with other chemicals such as pentachlorophenol (Amer

and Ali, 1969), amitrole (Mohandas and Grant, 1972), isopropyl-N-phenyl carbamate (IPC) and duphar (Amer and Farah, 1976) and Leptophos (Amer and Farah, 1979). In this connection Sparrow and Singleton (1953) mentioned that micronuclei are a fair index of fragment production. Most of the fragments lead to the formation of micronuclei. Micronuclei are true mutagenic effects (Auerbach, 1962), which may lead to the loss of the genetic material.

Micronuclei may araise due to the action of pesticides on the spindle apparatus, leading to the unequal separation of chromosomes at anaphase. The larger groups of daughter chromosomes form a comparatively larger nucleus and the smaller group forms a micronucleus.

Micronucleus may originate from a lagging chromosome at anaphase or from a chromosome fragment (Badr and Ibrahim, 1987). It seems probable that the micronucleus is derived from a whole lagging chromosome having higher probability to survive and undergo condensation in synchrony with the main nuclei than micronuclei derived from a chromosome fragment (Gustavino *et al.*, 1987). Micronuclei are true mutagenic aspects, which may lead to a loss of genetic material and have been regarded as an indication of mutagenicity of their inducers (Ruan *et al.*, 1992).

Hence the presence of micronuclei observed in the present study is not a deviant phenomenon. These are evidently the resultant of the chromosome fragments of the cells concerned. They may also arise from the lagging chromosomes.

Binucleate cell: - The formation of binucleate cells during interphase was observed in several experiments conducted with pesticides (Tables 1, 2, 3, 4, 7, 10; Figs. 15, 16, 17, 72, 73). Oksala and Therman (1974) as well as Graham *et al.* (1978), suggested that binucleate and multinucleated conditions are the peculiarities of cancer cells. Bovery (1974) stated that malignant tumours arise as

a result of a typical distribution of chromosomes through aberrant mitosis and this came to be known as "Chromosome theory of cancer". Delay or failure of cytokinesis would account for the occurrence of binucleate and multinucleate cells (Ene and Emadi, 1987). Binucleate cells are found due to the inhibition of cell wall development during telophase. Arrest of cytokinesis leads to the formation of binucleate and multinucleate cells (Kihlman, 1975). The formation of multinucleate cells may be the result of preceding multipolar mitosis or failure of cell plate formation (Grant, 1978). Binucleate cell may arise due to the suppression of cell plate formation in the early telophase by the pesticides. Hence neither the cell plate nor the cell wall appeared at the equatorial plane in the treated cells (Sato and Tanaka, 1972). Several cyclic hydrocarbons, methylated oxypurines and plant extracts were known to inhibit cell plate formation (Amer *et al.*, 1971).

Mechanism of cytokinesis in plant cells involves the formation of new cell walls with the help of different organelles like microtubules, golgi bodies and possibly mitochondria. Occasionally incomplete cell wall formation was also noticed. Such effects indicate that the chemicals found in the pesticides affect either the function of microtubules or golgi bodies or both. This might have led to the formation of binucleate cells.

Nuclear extrusion: - Nuclear extrusion is an abnormality present in both plant materials (Tables 1, 6; Figs. 20, 74). Due to the action of certain chemicals present in the pesticides Ethion and Metacid, the division stages were arrested and nuclear extrusion occurred. Raghuvanshi and Singh (1976) noticed such an effect with gamma rays. The cells may also show nuclear extrusion due to the karyokinetic activity of pesticides.

Nuclear deformation: - Interphase cell with deformed nuclei were observed after treatment with different concentrations of the pesticides Malathion and

Rogor (Tables 4, 9; Figs. 19, 75). The inhibition of cell division and the presence of deformed nuclei may be due to the action of the organophosphorus pesticides used (Morrison *et al.*, 1981). Deformed nuclei became vacuolated and elongated often curved or coiled. They can be considered as restitution nuclei formed after abnormal nuclear division (Chauhan and Chauhan, 1999). The above-mentioned types of abnormalities were observed in the mitosis of *Vicia faba* after treatment with other organophosphorus insecticides Leptophos, Dursban and Methamidophos (Amer and Farah, 1979). The chemical constituents present in the pesticides Malathion and Rogor seem to be responsible for nuclear deformation.

Sickle shaped nucleus: - Sickle shaped nucleus was present in the treatment of *Allium cepa* root tip cells with Metacid (Table 5; Fig. 11). It may be due to the disintegration of chromatin material by the direct action of chemicals present in the pesticides on the nucleus.

Nuclear fragments: - Nuclear fragments were observed in the treatment of *Allium cepa* root tip cells with Metacid and Rogor (Tables 5, 9; Figs. 13, 14). Amato (1950) observed fragmentation in large number and the percentage measured with increasing concentration and time. Fragmentation is the result of random breaking at different sites of the nucleus. Sharma (1980) pointed out that changes brought about in the viscosity of cytoplasm are responsible for nuclear aberrations. The used pesticides may affect the viscosity of cytoplasm and it may affect the nucleus and thus nuclear fragments were formed in the cytoplasm. Nuclear fragments might have formed due to the stickiness of the chromosomes and consequent failure of the separation of chromatids to poles. Bruhin and Warner (1954) also reported similar results. Hence it seems probable that these factors either singly or in combination may be responsible for nuclear fragments.

Elongated nucleus and dumbbell shaped nucleus: - Another interesting feature in the treated cells of *Allium cepa* were the occurrence of elongated nucleus and dumbbell shaped nucleus. In this study, the pesticide Malathion and Rogor induced elongated and dumbbell shaped nucleus (Tables 3, 9; Figs. 10, 18). The toxic chemicals present in the pesticides seem to be responsible for changing the viscosity of the cytoplasm, there by leading to the formation of elongated and dumbbell shaped nuclei.

Of these many mitotic abnormalities produced, some have very important significance also. Based on the percentage of abnormality, the genotoxicity of the five organophosphorus pesticides are compared and ranked. In *A. cepa*, Ethion induces the maximum genotoxic effect followed by Metacid, Nuvan, Malathion and Rogor. Where as in *A. sativum*, Metacid was found to be the most potent genotoxic agent followed by Ethion, Malathion, Rogor and Nuvan. It has been pointed out by a number of workers that abnormalities involving chromosome structure serves as elegant indicators of mutations. The number of structural abnormalities and ploidy changes observed in the present study point out to the fact that the toxic chemicals present in the applied pesticides can act as potent mutagenic agents. Grant (1978) pointed out that plant chromosomes are sensitive indicators to environmental pollutants and rightly suggested that the higher plant system appears to be an excellent indicator of the cytotoxic, cytogenetic and mutagenic effects of environmental mutagens. Therefore the plant system must be accepted as a first tier assay system for detection of the possible genetic damage resulting from the use of hazardous environmental chemicals. Grant (1978) has also shown that an excellent correlation exists between the frequency of both chromosomal abnormalities and c-mitosis. In the present investigation the clastogenic and nonclastogenic (turbogenic) aberration seem to occur simultaneously as if they are highly correlated. So it is high time that use of these

toxic substances should be reduced/banned. Indiscrimaminate use of these pesticides would cause irreparable chromosome damage in nontarget organisms especially human beings. Moreover it is also proved from the earlier investigations that *Allium* has an excellent correlation with mammalian systems (Grant, 1982). Hence it seems that the mammalian systems are easily susceptible to the genotoxic potential of these organophosphorus pesticides. Hence these toxic pesticides should be immediately replaced by other ecofriendly biological/herbal pesticides.

B. BIOCHEMICAL EFFECTS

Biochemical studies of various treatments of organophosphorus pesticides reveal the potential to produce genotoxic changes in DNA, RNA and protein content in *Allium cepa* and *Allium sativum*. Ethion, Malathion, Metacid, Nuvan and Rogor can cause the reduction in DNA, RNA, buffer soluble protein and total free amino acid synthesis (Tables 11 to 50, Figs. 125 to 140). Previous reports suggest that organophosphorus pesticides are capable of inhibiting the macromolecular biosynthesis (Chauhan and Sundararaman, 1990) and are known to affect biochemical processes in the cell (Liang *et al.*, 1967). The reduction in the amount of DNA and RNA content was directly proportional to the time and concentrations of these pesticides (Tables 11-20). Treatment with these organophosphorus pesticides affected pre-M phase (DNA, RNA and protein synthesis) and M-phasic period (chromosome and spindle movements, formation and function).

Normal function of these cells are suppressed either to do impairment of DNA synthesis or blockage at different stages of cell cycle (Amato, 1952). Cell cycle consists of interphase and mitotic phase. During S-phase, DNA replication takes place. DNA content becomes doubled. But our study proved that after the treatment of organophosphorus pesticides, the DNA content is reduced. Based on

the decrease in the amount DNA in *A. cepa* and *A. sativum*, the genotoxic effects of the pesticides were compared at the molecular level and ranked. Malathion, followed by Ethion, Rogor, Nuvan and Metacid, caused the maximum inhibition of DNA synthesis in A. cepa. Where as in *A. sativum* Nuvan, followed by Ethion, Rogor, Metacid and Malathion, induced maximum decrease in DNA content. Decrease in the DNA content may be due to a depression in the incorporation of nucleotides as a result of pesticide treatment (Swietlinska *et al.*, 1973). It is also possible to suggest that the reduction in the mitotic activity was due to the inhibition of DNA synthesis, which was considered as one of the major prerequisite for a cell to divide. The initiation of DNA replication marks the transition from G_1 phase to the period of S phase. During S phase, the total content of DNA increases from the diploid value of 2n to the fully replicated value of 4n. During G_2 phase, the cell has two complete diploid sets of chromosomes. The S phase was also called as the synthetic period when DNA is replicated, G_1 and G_2 standing for the two "gaps" in the cell cycle when there is no DNA synthesis (Lewin, 1997). Changes in all cell division activities brought about by the influence of pesticides will affect the nucleic acid and protein content in them (Schultz and Funderburk, 1967).

Taylor *et al* (1962) suggested that the chromosomal lesions are due to interruption in DNA replication in cells, which are just finishing the phase of synthesis. These cells reach division with gaps in their chromosomes presumably the torsions and tensions produced by chromosome coiling at prophase and anaphase movements often result in complete fragmentation. Davids (1973) reported mitotic index inhibition was accompanied by DNA synthesis inhibition. Swietlinska *et al.* (1974), Adam (1975), Mitchison (1971), Datta and Chedda (1980), Pandita (1986) and Yonus *et al.* (1988) also reported similar relations.

In the present investigation a severe decrease in the DNA content was observed in *A. cepa* after treatment with Ethion and Malathion, when compared with other pesticides (Figs. 125, 126). Where as in the case of *A. sativum*, all the pesticides studied decreased the DNA content in most of the treatment (Figs. 127, 128). It should be emphasized that genotoxic effects observed with Malathion may in fact arise from impurities in the technical grade mixtures. Highly toxic compounds increase in Malathion during storage due to time and heat. These impurities were found to weaken ability of lever to detoxify in human beings. These impurities have been identified (trimethyl phosphorothioates) and have been shown to behave like isoMalathion in potentiating the toxicity of Malathion. Malathion is able to literally "knock-off" genes from DNA molecules, there by leading to a decrease in the DNA content. However, the slight increase in the DNA content than control, which was observed in *A. cepa* after some treatments with lower concentration of Metacid (Tables 15; Figs. 125, 126) and in *A. sativum* at the lowest dose and duration (Table 16; Figs.127, 128) may be due to the "duplication effect" of genes induced by this organophosphorus pesticide under specific conditions. In addition to this, the increase in the DNA content induced by the above mentioned treatments with Metacid in *A. cepa* and *A. sativum* shows an inverse relation ship with the mitotic index in the respective treatments with Metacid (Figs. 117, 118, 119, 120). A drop in the mitotic activity, which is not accompanied by the inhibition of DNA synthesis, may be due to an increase in the G_2 period (Vant'Hoff, 1968).

In the present investigations the RNA content of both *A. cepa* and *A. sativum* were found to decrease after treatment with the five pesticides (Tables 21-30; Figs. 129-132). Depending upon the ability to inhibit RNA synthesis in the two test materials, the pesticides were compared and ranked. In *A. cepa*, the

maximum decrease in RNA content was induced by Malathion, followed by Nuvan, Ethion, Rogor and Metacid.Where as in A. sativum also maximum decrease in RNA content was shown by Malathion, followed by Nuvan, Rogor, Metacid and Ethion.. An earlier report suggests inhibition of RNA synthesis after treatment with pesticides (Mann et al., 1967). Similar relationship had also been established by several other workers like Lefler *et al.* (1971), Tomkins and Grant (1972), Davids (1973), Fishbein (1976), Momparler and Myronkaron (1976) and Badr and Ibrahim (1987). In plants, most of the phenolic herbicides inhibit oxidative phosphorylation (Weinbach, 1956; Gaur and Beevers, 1959; Kandler, 1960). The inhibition of oxidative phosphorylation results in inhibition of cell division (Kihlman, 1966). This is because the development of the mitotic process requires energy. Most of the energy required by the cell is provided by oxidative phosphorylation in mitochondria in the form of ATP. Epel (1963) has shown that the rate of mitosis in the sea urchin egg is closely related to the ATP level. Amer and Ali (1969) has also been noted the decrease in division frequency. This inhibition in DNA, RNA and protein synthesis might be due to the reduction of oxidative phosphorylation in plants resulting in the lowering of ATP levels. Significant decrease in sodium and potassium ATPase activity, suggest the disruption of ion transport processes in intestine after aerial spraying of Malathion in children (Wali *et al.*, 1984).

The organophosphorus pesticides with lethal and sublethal doses greatly reduce the DNA, RNA, protein and DNA/RNA ratio of fat body and silk gland in comparison to those of control worms of *Bombyx mori*. It is presumed that these pesticides causes major changes in nucleic acid content of silk worms (Surendra Nath *et al.*, 1996). Chromosomal aberrations have long been known to be associated with various types of mental retardation and morphological abnormalities in man (Peter, 1993). Malathion was found to cause DNA

abnormalities and decrease DNA content at all doses studied in human blood cells (Balaji and Sasikala, 1993). Turtle frogs have been reported to be very susceptible to developing mutations from the pesticide Malathion. Nuvan can bind to molecules such as DNA and several studies have shown dichlorvos to be a mutagen.

Ignacimuthu and Babu (1988) pointed out that the decrease in mean DNA content per cell is directly proportional to the decrease in the rate of RNA turn over. Such an induced decrease in RNA synthesis has been reported in irradiated seeds of lettuce also. Yearly and Stone (1975) and Mann *et al.* (1967) studied that isoproturon affects diffused chromatin and inhibited RNA synthesis in the root meristem cells of *Allium cepa.* Ashton and Crafts (1973) stated that all herbicides that reduced ATP levels are strong inhibitors of RNA and protein synthesis. Metabolic disturbances due to the effect of chemicals generally lead to the inhibition of cell division, which in turn believe to be due to the effect on the synthesis of DNA, RNA, protein and energy (Hess, 1983). The inhibitions of DNA, RNA and protein synthesis with 2,4-dinitrophenol treatments have been noted by using tritium labelled precursors. The effect was more drastic at higher concentrations. Thus the decrease in division frequency by the phenolic herbicides has some correlation with the inhibition of DNA synthesis. This inhibition in DNA, RNA and protein synthesis by 2, 4-dinitrophenol might be due to the reduction of oxidative phosphorylation in plants resulting in the lowering of ATP levels (Chand and Roy, 1981). The herbicide Glean remarkably inhibited mitotic division and depressed DNA and RNA in root meristems of *Allium cepa and Vicia faba* (Badr and Ibrahim, 1987). Many of the urea herbicides have been reported to give positive response in DNA synthesis inhibition (Sieler, 1978).

Isoproturon affects defused chromatin and inhibits RNA synthesis in the root meristem cells of *Allium cepa.* In this regard it is important to mention that number of other pesticides have also been shown to induce chromatin condensation and inhibit RNA synthesis (Yoshida *et al.*, 1983; Mann *et al* 1967). Inhibition of RNA synthesis is associated with the decrease in germination due to mutagen treatments, which might have destroyed the ribosome; the radiation-induced inhibition of seed germination might be either due to destruction of ribosome (Van Huystee *et al.*, 1968) or because of ribosomal genes. Varner (1964) suggested that GA_3 enhances the RNA and protein synthesis, which in turn helps to repair the cytological damage caused by physical and chemical mutagens.

In the present study treatments of various organophosphorus pesticides cause the reduction in buffer soluble proteins (Tables 31- 40; Figs.133-136). It is directly proportional to the concentrations of the organophosphates applied. However, the slight increase in the buffer soluble proteins in *A. cepa* after treatment with the lowest concenteration of Metacid (Table 35) may be due to the already mentioned "gene duplication effect" under specific conditions. The five organophosphorus pesticides are compared and ranked based on their capacity to decrease the buffer soluble protein content in the test materials. In *A. cepa*, the maximum decrease in the protein content was induced by Ethion, followed by Rogor, Nuvan, Malathion and Metacid. Where as in *A. sativum* the maximum decrease in protein content was shown by Nuvan, followed by Ethion, Rogor, Malathion and Metacid. Several earlier workers envisaged a decrease in the content of proteins after treatments with pesticides (Hoffman-Berling, 1954; Butts and Fangs, 1956; Cummins et al., 1966; Schultz and Funderburk, 1967; Chand and Roy, 1981; Hess, 1983; George and Ghareeb, 2001). Adam *et al.* (1990) suggested that the general effect of Malathion and Tamaron in increasing

the percentage of metaphase, anaphase and telophase was accompanied by a rise in protein content of the treated root tips. This may be explained on the basis that spindle formation required active protein synthesis to form the microtubules (spindle sub units). Significant and dose dependent decrease of mitotic indices suggested mitodepressive action of these compounds. These metabolic disturbances may lead to a change in the cell divisions, which may cause change in the synthesis of DNA, RNA, protein and energy (Hess, 1983).

Application of organophosphorus pesticides causes the clastogenetic abnormalities like C-metaphase. It is the cytological effects of the inhibition of protein synthesis during mitosis. Adriamycin binding prevents DNA from unwinding for transcription of spindle protein messengers (Mercykutty and Stephen, 1980). Thus in the present investigation it seems probable that the organophosphate binding prevents the DNA from unwinding for transcription, there by leading to a decrease in the RNA and protein content in the treated root tissues. The impact of pesticide pollution on the fresh quality of a few fish species was studied. The result shows a significant decline in the extractable protein content (Kaur and Saxena, 2001). Acetylcholine esterase activity decreased at all the dose levels of dichlorvos in *Rattus norregiciss* (Praveen and Santhosh, 2001). Effect of urea fertilizer application on silver leaf white fly was examined. It affected levels of soluble proteins in cotton petioles (Bi *et al.*, 2003).

Organophosphates like Ethion cause decrease in the protein content of silk worms (Surendra Nath *et al* 1996). GA can cause inhibition of protein synthesis, which in turn causes inhibition of RNA synthesis and unbalanced DNA synthesis (Maaloe and Kjeldgaard, 1966). Stickiness has also been attributed in the reaction of the applied pesticides with DNA or protein or both of them forming inter and intra chromosomal cross-links (Patil and Bhat, 1992).

In the present investigation, decreased amount of amino acid content was observed after the treatment with Ethion, Malathion, Metacid, Nuvan and Rogor (Tables 41-50; Figs.137-140). The decrease in the total free amino acids was observed both in *A. cepa* and *A. sativum*. Based on the potentiality to decrease the amount of total free amino acids, the five organophosphorus pesticides were compared and ranked. In *A. cepa*, Rogor, followed by Ethion, Malathion, Metacid and Nuvan, brought about the maximum decrease in amino acid content. Where as in *A. sativum*, Nuvan induced maximum decrease in the amount of amino acids, followed by Malathion, Ethion, Metacid and Rogor. Several pesticides were found to affect the biochemical processes in the cell there by causing drastic decrease in the amount of amino acids in the cells (El-Shehaby and Mohamed, 1985; Rost and Reynold, 1985). However the profound decrease in amino acid content when *A. sativum* was treated with Nuvan (Table 48; Fig. 139) and slight increase in amino acid content when *A. sativum* was treated with Metacid (Table 46; Fig. 139) and *A. cepa* was treated with Metacid (Table 45; Fig. 137) may be due to disturbances in amino acid profile caused by the toxic chemicals in the pesticides concerned. Chronic exposure to mixtures of organophosphorus pesticides leads to disturbances of amino acid profile (Gomes *et al.*, 1999).

In vivo study of Carbofuran exposure cause the alteration in free fatty acid content in different tissues of fish (Beegum and Shantha, 2001). Changes in total protein and free amino acids in the ovaries of two fresh water fishes by subjecting them to increasing sub lethal concentration and duration of exposure to carbamates were studied (Kamble *et al.*, 2002).

It may be concluded that the cell division is an energy dependant process and there by the movement of chromosomes mainly depends upon the energy generating system. According to Swann (1954) energy is steadily released by

respiration and stored in the form of some organic compounds and is utilized in starting the division. The pesticide treatment somehow disturbed the respiratory pathways, resulting in the low accumulation of energy containing and other essential compounds-ATP, sugar and protein molecules etc. Epel (1963) reported that the rate of mitosis was closely related to the resultant level of ATP and mitosis could be blocked at any stage at appropriate time by adding optimum concentration of respiratory inhibitors.

The present study reveals that the applied organophosphorus pesticides caused more hindrance in chromosomes movement. Vant' Hoff (1968) suggested that the inhibition of mitotic activity by chemical compounds is due to an increase in the G_2 period. Webster and Davidson (1969) and Mac Leode (1969) attributed the inhibition of mitosis to the increase in S-phase duration. Cytological examination of dividing cells revealed an abundance of dose dependant chromosome irregularities. The production of chromosomal abnormalities provides a valuable genetic assay for screening environmental pollutants (Grant, 1994). However, the dominant irregularity induced by these toxins was found to be chromosomal stickiness, which is a physiological, and transient phenomenon induced in dividing cells by numerous chemical compounds (El-Shazly, 1995). Stickiness is considered as a mitotic disruption that is not likely to lead to chromosomal structural damage (Badr *et al.*, 1995).

The present study clearly reveals that the hereditary constitution of an organism may be changed as a result of pesticides treatments. Such changes occur in nature following the use of pesticides and may have far reaching implications in the maintenance of pure seed lines, resistance of organisms to pesticide and evolutionary change of biota. Furthermore, the present study suggests that long exposure to the pesticides or accumulation of pesticides in the environment as pollutant may cause spontaneous chromosomal anomalies in the

domestic animals and human beings also. Therefore, there is a great need to make routine survey of different localities to examine the chromosomal behaviour in the plant community in order to ascertain the accumulation of pollutants at particular places. This would prove to be the cheapest and simplest method to detect the intensity of pollution. The greater the spontaneous chromosomal anomalies, the greater would be the accumulation of pollutants at that particular place. Moreover, *A. cepa* assay has excellent correlation with mammalian systems (Grant, 1982).

Similarly plant chromosomes are sensitive indicators to environmental pollutants and rightly suggested that the higher plant systems appears to be an excellent indicator of the cytotoxic, cytogenetic and mutagenic effects of pesticides. Therefore the plant system must be accepted as a first tier assay system for detection of the possible genetic damage resulting from the use of environmental chemicals.

A large number of pesticide chemical agents are reported as mutagenic and carcinogenic agents to genetic material. These organophosphorus pesticides are also leading pollutants. Exposure to these environmental chemicals is suspected of causing several serious ailments in human body, including genetic disorders and neuron developmental defects. The hazardous effects of these chemical agents have a great potential for disturbing the ecological balance. The toxicants that act on DNA cause damage to the genome and induce alterations in the nucleic acids and result in the modification or inactivation of a cell's genome. It is also recognized that many chemicals in our environment are potential causes of cancer and /or mutagenic. Mutagens of environmental significance have gained special attention on account of severe environmental pollution and possible genetic hazards after their leakage.

All these results indicate that the used pesticides have cytotoxic properties and even can act as a mutagen. Thus it brings about various effects that are brought about by other mutagenic agents. Due to ignorance and over ambitions of farmers in many developing countries, there is every likelihood of applying the insecticides in higher concentrations than the recommended doses. These may cause some cytological disturbances, which lead to genetic change. The appearance of multivalent and chromatin bridges suggest that these chemicals can affect genetic recombination, which may lead to the loss of important factors or gain undesirable characters. It was also clear that their effects on chromosomes might lead to genetic changes in the plants although there were no morphological differences. The routine cytogenetic screening of standing crops may be one of the helping tools in maintaining the purity of germplasm. It is worthwhile mentioning that farmers should be warned against the usage of these insecticides over and above the prescribed concentrations. The necessity for screening the agrochemicals for their possible mutagenic potentialities that are detrimental to the better economic utilization of plants is suggested.

The differences observed in the cytotoxicity and the amounts of biochemical molecules between *A. cepa* and *A. sativum* suggest that these effects are organism specific. This may be due to the change in the duration of the mitotic cycle and variation in DNA amounts, which results in substantial interspecific variation in *Allium* (Evans et al., 1972). In conclusion, it can be stated that the cytotoxic and biochemical effects of the five organophosphorus pesticides studied are concentration and time dependant and even test material dependant. So it is advisable to stop or limit the use of Ethion, Malathion, Metacid, Nuvan and Rogor for plant protection and suggest the use of alternate form of eccofriendly pesticides.

SUMMARY

Kochuthressia M. V. "Cytotoxic and biochemical effects of some organophosphorus pesticides in Allium cepa L. and Allium sativum L." Thesis. Department of Botany, University of Calicut, 2005

SUMMARY

In the present investigation, cytotoxic and biochemical effects of five organophosphorus pesticides Ethion, Malathion, Metacid, Nuvan and Rogor had been analyzed in *Allium cepa* and *Allium sativum* root tip meristem. All the pesticides were capable of inducing various types of mitotic aberrations, increased percentage of abnormalities and decreased mitotic index. The applied pesticides caused decreased amounts of DNA, RNA, buffer soluble protein and total free amino acids.

CYTOTOXIC EFFECTS

Cytotoxicity of various concentrations (0.0125%, 0.025%, 0.05%, 0.1%) of pesticides Ethion, Malathion, Metacid, Nuvan and Rogor at different time durations (one and two-hours) on *Allium cepa* and *Allium sativum* root tip meristems were analyzed. The frequency of chromosomal aberrations induced by these pesticides deviated significantly from that of the control. Treatment with pesticides for one and two-hours caused severe cytotoxicity and distinct decrease in the mitotic index indicating mitotic inhibition. In *Allium cepa* the maximum mitodepressive activity was induced by Rogor followed by Nuvan, Metacid, Malathion and Ethion. Where as, in *Allium sativum* Rogor caused severe mitotic depression followed by Metacid, Nuvan, Ethion and Malathion. The extent of cell cycle delay was generally correlated with the dose of an organophosphorus pesticide. The higher the dose, the more the delay. The cytotoxic effects of all pesticides include many clastogenic and nonclastogenic abnormalities. The major nonclastogenic abnormalities observed include misorientation of chromosomes, highly irregular chromosomes at metaphase, dislocation of chromosomes, chromosome clumping, chromosome pulverization, ball metaphase, star meta/anaphases, diagonal orientation of chromosomes, C-metaphase, unequal segregation and abnormal association of chromosomes, oblique metaphase, scattering of chromosomes, polyploidy, multipolarity, stathmo anaphase, early

movement, lagging chromosomes, disturbed pro-meta-ana-telophases, elongated nuclei, dumbbell shaped nuclei, nonsynchronous movement, micronuclei, binucleate cells etc. The clastogenic aberrations include chromosome stickiness, chromosome fragmentation, chromosome erosion, chromosome pulverization, chromosome bridges, ring chromosome, nuclear fragments, nuclear deformation, nuclear lesions, sickle shaped nucleus etc.

Of these many nuclear abnormalities produced, some have very important significance. Of the pesticides tested in both systems, those showing cytotoxic activity in *Allium cepa* were also cytotoxic in *Allium sativum*. In *Allium cepa*, Ethion exerts the maximum genotoxic effect followed by Metacid, Nuvan, Malathion and Rogor. Where as in *Allium sativum* Metacid shows the maximum genotoxicity followed by Ethion, Malathion, Rogor and Nuvan. It has been pointed out by a number of workers that abnormalities involving chromosomes structure serves as an elegant indicator of mutations. Number of structural abnormalities and ploidy changes observed in the present study point out that the chemicals present in the applied pesticides may behave as potent mutagenic agents.

BIOCHEMICAL EFFECTS

Besides the mitoclastic and turbogenic effects the present study gives a variety of biochemical changes in *Allium cepa* and *Allium sativum*. The results indicate that these pesticides act as an inhibitor of cell cycle progression at the G_1 transition point. The inhibition of mitotic activity induced by pesticide treatments is associated with a reduction in the amount of DNA, RNA, buffer soluble protein and total free amino acids. Cytological study reveals that significant decline occur in mitotic indices. This metabolic change causes the alteration in cell divisions. This in turn results in change in the content of DNA,

RNA, protein and amino acids. C-metaphase and delayed metakinesis are the cytological effects of the inhibition of protein synthesis.

The five organophosphorus pesticides may be ranked based on their capacity to induce decrease in the amount of biochemical molecules.

I. Based on inhibition of DNA synthesis in *A. cepa*, these five pesticides may be ranked as Malathion>Ethion>Rogor>Nuvan>Metacid.

II. Inhibition of DNA synthesis in *A. sativum*: -
Nuvan>Ethion> Rogor>Metacid>Malathion.

III. Decrease of RNA content in *A. cepa*: -
Malathion >Nuvan>Ethion>Rogor>Metacid.

IV. Decrease of RNA content in *A. sativum*: -
Malathion >Nuvan>Rogor>Metacid>Ethion.

V. Lowering of buffer soluble protein content in *A. cepa*: -
Ethion> Rogor>Nuvan>Malathion>Metacid.

VI. Lowering of buffer soluble protein content in *A. sativum*: -
Nuvan> Ethion>Rogor>Malathion>Metacid.

VII. Decrease in total free amino acids in *A. cepa*: -
Rogor>Ethion>Malathion>Metacid>Nuvan.

VIII. Decrease in total free amino acids in *A. sativum*: -
Nuvan>Malathion>Ethion>Metacid>Rogor.

Potent DNA inhibitors are primarily responsible for reduction in mitotic activity or it could be due to inhibition of DNA synthesis or change in the oxidative phosphorylation activity. The drastic effect of pesticides in the present study could be due to its ability for immediate binding and reactivity at many sites that could be considered analogous to 'prophase poisoning.' It is thus inferred that normal function of the cell is suppressed either due to impairment of DNA synthesis or blockage of synthetic stage of interphase cells.

The chemicals used in the present study are alkylating agents and therefore could be mutagenic or carcinogenic which are chemically reactive. The chromosome fragments rendered by different treatments did not show any reunion of broken parts. It may be assumed that chemicals present in these pesticides besides producing extensive chromosomal aberrations may simultaneously interfere with the normal functioning of repair enzyme and series of actions involved in the process of rejoining.

The present study clearly reveals that the hereditary constitution of an organism may be changed as a result of pesticide treatments. Such changes occur in nature following the use of pesticides and may have far reaching implications in the maintenance of pure line, resistance of organisms to pesticide and evolutionary change of biota. Further more, the present study suggests that long exposure to the pesticide or accumulation of pesticides in the environment as pollutant may cause spontaneous chromosomal anomalies in the domestic animals and human beings also. The greater the spontaneous chromosomal anomalies, the greater would be the accumulation of pollutants.

Continuous exposure of these pesticides or continuous conception of pesticide sprayed food products may also cause some genetic disorders in human beings. When we loose too many genes in the cells controlling one part of our health, we become 'genetically weak' since the cells cannot operate at high efficiency to do their job. It is a matter of balance. Once our cells become so inefficient because of the gene loss, then we too can die. The lesson to be learned from this is that we do not want to expose ourselves to pesticide or chemicals that can accelerate gene loss in important cells which are protecting us from bacteria, viruses etc.

REFERENCES

Abraham, K. O. (1976). Studies on sulphur flavour constituents' in asafoetida and *Allium*. Ph.D thesis, University of Mysore, Mysore, India.

Abraham, S. (1965). Studies on spontaneous and induced mutations in plants. **Cytologia** 30: 155-172.

Abraham, S. (1997). Studies on cytological changes induced by muriate of potash in *Allium cepa*. **Cytologia** 62: 291-294.

Abraham, S. and Cherian, V.D. (1976). Somatic cell abnormalities induced by water extracts of *Gloriosa superba*. **Nucleus** 19: 17-20.

Abraham, S. and Devi, C. K. V. (1989). Inhibition of cytokinesis and induction of mixoploidy by magnesium sulphate. **Caryologia** 42: 121-126.

Adam, Z. M. (1975). Morphological effects of some nematicides. Ph.D. thesis, Aim Shams University, Cairo- Egypt.

Adam, Z. M., Ebab, F. A. and El-Shaikh, I .A. (1990). Alterations in nucleic acid, protein content and mitotic division of *Vicia faba* root tip cells as effected by Malathion and Tamron insecticides. **Cytologia** 55: 349-355.

Ahmad, S. and Yasmin, R. (1992). Effects of methyl parathion and Tri-miltox on the mitosis of *Allium cepa*. **Cytologia** 57: 155-160.

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

Airapetyan, R. B. (1986). Cytogenetic activity of the herbicides 2,4-D, Dalapon and Semeron on chromosomes of *Allium cepa* L. **Biologicheskii-Zhurnal-Armenii** 37: 404- 408.

Airapetyan, R. B. (1987). Cytogenetic activity of some insecticides on the chromosome apparatus of onion. **Biologicheskii-Zhurnal-Armenii** 40: 163-164.

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

Amato, F. D. (1948). The effects of colchicine and ethylene glycol on sticky chromosomes in *Allium cepa*. **Hereditas** 34: 83-103.

Amato, F. D. (1949). Gammaxene as a polyploidizing agent. **Caryologia** 1: 209- 232.

Amato, F. D. (1950). Notes on the chromosome break induced by pure gammaxene. **Caryologia** 2: 361- 364.

Amato, F. D. (1952). Polyploidy in the differentiation and function of tissues and cells in plants. Critical examination of literature. **Caryologia** 4: 311-357.

*Amer, S. M. (1960). Vergleichende untersuchungen uber die Wirkung stathmokinetischen stoffe (Dissertation, Munchen).

Amer, S. M. (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. (1968). Cytological effects of pesticides II. Meiotic effects of some phenols. **Cytologia** 33: 21-33.

Amer, S. M. and Ali, E. M. (1969). Cytological effects of pesticides IV. Mitotic effects of some phenols. **Cytologia** 34: 533-540.

Amer, S. M. and Ali, E. M. (1986). Cytological effects of pesticides XVII. Effect of the insecticide dichlorvos on root mitosis of *Vicia faba.* **Cytologia** 51: 21-25.

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

Amer, S. M. and Farah, O. R. (1976). Cytological effects of pesticides VIII. Effect 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 phosphorothioate insecticide Leptophos on *Vicia faba*. **Cytologia.** 44: 241-245.

Amer, S. M. and Farah, O. R. (1983). Cytological effects of pesticides XII. Effect of the phosphorothioate insecticide Dursban on the mitosis of *Vicia faba*. **Cytologia** 48: 27-33.

Amer, S. M. and Farah, O. R. (1985). Cytological effects of pesticides XV. Effect of the insecticide Methamidophos on root-mitosis of *Vicia faba*. **Cytologia** 50: 521-526.

Amer, S. M. and Mikhael, E. (1986). Cytological effect of the insecticide Rotinone on root –mitosis of *Vicia faba*. **Cytologia** 51: 171-176.

Amer, S. M., Hammouda, M. A. and Farah, O. R. (1971). Cytological and morphological effect of insecticide N-methyl-1-naphthyl carbamate, "Sevin". **Flora** 160: 433-439.

Ames, B. N. (1971). The detection of chemical mutagens with enteric bacteria. In: Chemical Mutagens. A. Hollaender (ed.), Plenum Press, NewYork. Pp. 267 - 282.

Ames, B. N. and Mc Cann J. (1976). Carcinogens are mutagens. A simple test system In: Screening Tests in Chemical Carcinogenesis. R. Montesano, H. Bartsch. and L. Tomatis (eds.), IARC, Lyon. Pp. 493-501.

Ames, B. N., Burston, W. E., Yamasaki, E. and Lee, F. D. (1973). Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. **Proc. Natl. Acad. Sci. (U.S.),** 70: 2281-2285.

Ames, B. N., Mc Cann, J. and Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsome mutagenicity test. **Mutat. Res.** 31: 347-364.

Amita, G., Sant-Prakash Gupta, S. P., Gulati, A. and Prakash, S. (1994). Genotoxicity testing for relative efficacy of selected pesticides on *Allium cepa*. **Journal of Environmental Biology** 15: 89-95.

Amoore, J. E. (1961a). Arrest of mitosis in roots by oxygen lack or cyanide. **Roy. Soc. (London), Proc. B.** 154: 95-108.

Amoore, J. E. (1961b). Dependence of mitosis and respiration in roots upon oxygen tension. **Roy. Soc. (London), Proc. B.** 154:109-129.

Ann, H. N., Vienne, M. and Van Den Berghe, H. (1979). Induction of sister-chromatid exchanges in cultured human cells by an organophosphorous insecticide Malathion. **Mutat. Res.** 67: 167-172.

Araratyan, L. A. and Azatyan, R. A. (1974). Radioprotective effect of beta-indole acetic acid and its stimulating effect on DNA synthesis in cells of *Crepis capillaris* L. **Seed. Tritol Genet.** 8: 299-302.

Ashton, F. M. and Crafts, A. S. (1973). Mode of Action of Herbicide. Wiley, A. Inter Science Publication, New York.

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

Auerbach, C. (1962). Mutation – An Introduction to Research on Mutagenesis. Part I. Methods. Oliver and Royd, Edinburgh.

Ayonoadu, V. W. V. (1974). Nuclear DNA variation in *Phaseolus*. **Chromosoma (Berl)** 48: 41-49.

Babu, K. A., Shah, V. C. and Lakhotia, S. C. (1980). Effect of benzamide on mitosis and chromosomes in mammalian cells *in vitro*. **Ind. J. Exptl. Biol.** 18: 329-332.

Badr, A. (1983). Cytogenetic activities of Triazine herbicide in root tips of *Allium cepa* and *Vicia faba*. **Mutat. Res.** 117:173 -182.

Badr, A. (1988). Cytogenetic activities of some fungicides. **Cytologia** 53: 635-640.

Badr, A. and Elkington, T. T. (1982). Antimitotic and chromotoxic effects of isoproturon in *Allium cepa* and *Hordeum vulgare*. **Environ. Exp. Bot.** 22: 265-270.

Badr, A. and Ibrahim, A. C. (1987). Effect of herbicide Glean on mitosis, chromosomes and nucleic acids in *Allium cepa* and *Vicia faba* root meristems. **Cytologia** 52: 293-302.

Badr, A., Shehab, A. S., Khriralla, Z. H. and El-Shazly, H. H. (1995). Mutagenic potential of aflatoxin produced by *Aspergillus parasiticus* and its effect on growth and yield of *Vicia faba*. **Con. Toxiol. Dev. Count. Cairo, Egypt.** 19-23 Nov. preceeding Vol II Pp. 99-114.

Bajar, A. and Mole Bajar, J. (1969). Formation of spindle fibres, kinetochore orientation and behaviour of the nuclear envelope during mitosis in endosperm. Fine structural and *in vitro* studies. **Chromosoma** 27: 448-487.

Bajar, A. and Mole Bajar, J. (1972). Spindle Dynamics and Chromosome Movements. Academic Press, New York. Pp.131-157.

Balaji, M. and Sasikala, K. (1993). Chromosome damage occurs to human blood cells. **Mutat. Res.** 301: 13-17.

Bansal, Y. K. and Sen, S. (1979). Differential response of chromosomes of young and adult tissue to chemicals. **Current Science** 48 : 448-449.

Barber, H. N. and Callan, H. G. (1942). The effects of cold and colchicine on mitosis in the newt. **Proc. Roy. Soc.** 131: 258-271.

Barham, S. S. and Brinkley, B. R. (1976). Action of rotenone and related respiratory inhibitors on mammalian cell division. 1. Cell kinetics and biochemical aspects. **Cytobios** 15: 85-96.

Bartels, P. G. and Wolf, F. T. (1965). The effect of amitrole upon nucleic acid and protein metabolism of wheat seedlings. **Physiol. Plant.** 18: 805-812.

*Barthelmess, A. (1957). Chemisch indizerte multiplare mitosenentwieklung. **American Journal of Botany** 54: 945.

*Barthelmess, A. and El – Kabarity (1962). Chemisch Induzierte multipolar Mitosen II. **Protoplasma** 54: 455-475.

*Bartlett, B. R. (1964) Biological control of insect pests and weeds. De-Bach P. (ed.), Reinhold, New York, Chapman & Hall, London. Pp. 489 - 511.

Beck, B. and Obey, G. (1975). The human leucocyte test system. VI. The use of sister chromatid exchange as possible indicators for mutagenic activities. **Human Genetic** 29: 127-134.

Bedford, C. and Robinson, J. (1972). The alkylating properties of organophosphates. **Xenobiotica** 4: 307-337.

Beegum, G. and Shantha, V. (2001). Carbofuran toxicity on total lipids and free fatty acids in air breathing fish during exposure and cessation of exposure-*in vivo*. **Environ. Monit. Assess.** 70: 233-239.

Bellami, L. M., Rinallo, C. and Bennici, A. (1991). Cytomorphological alterations in *Allium* roots induced by surfactants. **Environ. Exp. Botany** 31: 179-185.

Bell, S. L., Sehwart, O. J. and Hughes, K. W. (1976). Studies of the herbicide paraquat. 1.Effect on the cell cycle and DNA synthesis in *Vicia faba.* **Can. J. Genet. Cytol.** 18: 93-99.

Bentham, G. and Hooker, J. D. (1880). "Genera Plantarum" Vol. III. L. Ruve and Co., London.

Beuret, E. (1980). Effect of Pendimethalin on root meristems in *Allium cepa* L. **Weed Research** 20: 83-86.

Bhattacharya, S. S. (1955). The influence of Systox, Metasystox and Pertox on the nuclei and chromosomes of *Vicia faba*. **Phytopath. Z.** 23: 233-248.

Bi, J. L., Toscano, N. C. and Madore, M. A. (2003). Effect of urea fertilizer applications on soluble protein and free amino acid content of cotton petioles in relation to silver leaf white fly (*Bemisia argentifolii*) population. **J. Chem. Ecol.** 29: 747-761.

Bloom, W., Zirkle, R. F. and Uretz, R. B. (1955). Irradiation of parts of individual cells III. Effects of chromosomal and extra chromosomal irradiation on chromosome movements. **Ann. N. Y. Acad. Sci.** 59: 503-513.

*Bovery, T. (1974). Zur Frage der Entstehung Malinger Tumoreu Gustan Fisher, Jena.

Bridges, B. A., Mottershead, R. P., Anne Rothwell, M. and Green, M. H. L. (1972). Repair deficient bacterial strains suitable for mutagenicity screening tests with the fungicide Captan. **Chem. Biol. Interactions** 5: 77-84.

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* and some derivatives deficient in DNA repair. **Mutat. Res.** 19: 295-303.

Brown, A. W. A. (1962-63). Effects of insecticide on wild life. **Conservationist** 17: 8-11.

Bruhin, A. and Warner, H. (1954). On the effect of insecticide on mitosis in plants. **Phytopath.** 22: 327-342.

Brusick, D. (1982). Genetic Toxicology. In: Principles and Methods of Toxicology. Hayer A.W. (ed.), Raven Press. New York Pp. 223 - 272.

Buiatti, M. and Ronchi, V. N. (1969). Effect of cycloheximide and chloramphenicol on 6-methyl-coumarin induced chromosome breakage in *Allium cepa*. **Caryologia** 22: 375-378.

Bullought, W. S. (1952). The energy relations of mitotic activity. **Biol. Rev.** 27: 133-168.

Burton, K. (1968). Determination of DNA concentration with diphenylamine. In: Methods in Enzymology. Vol.XII: Nucleic acids. (Part B) Grossman, L. and Moldave, K. (eds.), Academic Press Inc., New York. Pp. 163-166.

Butani, J. V. and Shukla, P. T. (1994). Cytological effects of pesticides on onion (*Allium cepa* L.) root tip. **Gujarat Agricultural University Research Journal.** 20: 60-65.

Butt, S. K. and Vahidy, A. A. (1994). Cytotoxic effects of herbicide Roustar on meristematic cells of *Allium cepa* L. **Pakistan Journal of Botany.** 26: 69-74.

Butts, J. S. and Fang, S. C. (1956). Tracer studies on the mechanism of action of hormonal herbicides. Conference on radioactive isotopes in agriculture, U.S.A. **Atomic Energy Commission, Report. No. TID** 7512: 209-214.

Calora, F. B. (1956). Three insecticides in the control of insects affecting low land rice. **Pesticides** IX: 520-527.

Carlson, J. G. (1938). Mitotic behaviour of induced chromosomal fragments backing spindle attachment in the neuroblasts of the grasshopper. **Proc. Nat. Acad. Sci.** USA 24: 500-507.

Carrano, A. V., Thompson, L. H., Lindl, P. A. and Minkler, J. L. (1978). Sister chromatid exchanges as an indicator of mutagenesis. **Nature** 271: 551-553.

Carter, S. K. (1975). Adriamycin-a review. **J. Natl. Cancer Inst**. 55:1265-1274.

Cavallito, C. J., Buck, J. S. and Sater, C. M. (1944). Allicin the antibacterial principle of *Allium sativum*. L. 1. Isolation, physical properties and antibacterial action. **J. Amer. Chem. Soc.** 66: 1950-1951.

Chacko, M. (1981). Genetic toxicology of antiamoebic drugs. M.Phil dissertation, University of Kerala, India.

Chand, S. and Roy, S. C. (1981). Effects of herbicide 2,4-dinitrophenol on mitosis, DNA, RNA and protein synthesis in *Nigella sativa* L. **Biol. Plant.** 23: 198 –202.

Chandra, B. R., Saharan, R. P. and Sareen, P. K. (2002). Clastogenic effect of Trifluralin in *Vicia faba*. **J. Cytol. Genet.** 3: 201-203.

Chauhan, L. K. S. and Sundararaman, V. (1990). Effect of substituted urea on plant cells II. Ultra structural effects of isoproturon on the root meristem cells of *Allium cepa*. **Cytologia** 55: 99-105.

Chauhan, L. K. S., Saxena, P. N. and Gupta, S. K. (1999). Cytogenetic effects of cypermethrin and fenvalerate on the root meristem cells of *Allium cepa*. **Environmental and Experimental Botany** 42: 181-189.

Chauhan, N. and Chauhan, A. K. S. (1999). Genotoxicity of fluoroquinolones in *Allium* test system. **J. Cytol. Genet.** 34: 153-160.

Chaurasia, O. P. and Sinha, S. P. (1986). Cytogenetic effects of superphosphate in onion root tip cells. In: Perspectives in Cytology and Genetics. Manna, C.P. and Sinha, V. (eds.) Vol. V, Pp. 349-352.

Chen, H., Sirianni, S. and Huang, G. (1981). Sister chromatid exchanges in Chinese hamster cells treated with seventeen organophosphorus compounds in the presence of a metabolic activation system. **Enviro. Mutagenesis** 4: 621-624.

Clarke, C. H. (1971). The mutagenic specification of pentachloronitrobenzene and Captan, two environmental mutagens. **Mutat. Res.** 11: 247-248.

Clark-Walker, G. D. and Linnene, A. W. (1966). *In vivo* differentiation of yeast cytoplasmic and mitochondrial protein synthesis with antibiotics. **Biochem. Biophys. Res. Commun.** 25: 8 – 13.

Cook, J. (1967). Review of the Present Safety Arrangements for the Use of Toxic Chemicals in Agriculture and Food Storage. Second revised edition, Springer verlag, Berlin, New York.

Cottam, C. (1965). The ecologist's role in problems of pesticide pollution. **Biol. Sci.** 15: 457-463.

Cummins, J. E., Bloomquist, J. G. and Rusch, H. P. (1966). Anaphase delay after inhibition of protein synthesis between late prophase and pro metaphase. **Science** 154: 1343-1344.

Darlington, C. D. (1942). Chromosome chemistry and gene action. **Nature** 149: 66-69

Darlington, C. D. and Mc Leish, J. (1951). Action of maleic hydrazide on cell. **Nature** 167: 407-408.

Das, N. T., Raj S. A and Rao, R. B. V. (1968). Cytological studies in *Vicia faba*, L. treated with asafoetida. **Cytologia** 33: 100-111.

Datta, S. P. and Chedda, G. B. (1980). Synthesis and property of N-carbamoyl derivatives of cytosine, cytidine, uracil and thymine. **J. Carbohydrates Nucleosides Nucleotides** 7: 217-240.

Datta, A. K., Ranjit Chattopadhyay, S., Alokanada B., Saurav Chattopadhyay, S., Mukherjee, S., Chattopadhyay, R. and Biswas, A. (1995). Genotoxic effects of some pesticides and fungicides in three plant species. **Environment and Ecology.** 13: 633- 636.

Davids, I. (1973). Antimitotic activity of methyl benzimidazole-2yl carbamate (MBC) in *Aspergillus nidulans*. **Pestic. Biochem. Physiol.** 3: 317-375.

Dawey, M. C. and Miller, H. H. (1969). X-ray induction of chromatid exchanges in mitotic and G_1 Chinese hamster cells pretreated with colcemid. **Exp. Cell Res.** 57: 63-70.

Dean, B. J. (1972). The mutagenic effect of organophosphorus pesticides on microorganism. **Arch. Toxikol.** 30: 67-74.

Dedek, W., Lohs, K. H. and Sehmidt, R. (1976). Alkylation of guanine in mice *in vitro* by organophosphorus insecticides. 1.Trichlorfon and butonate. **Pest. Biochem. Physiol.** 6: 101-110.

de-Kergommeaux, D. J., Grant W. F. and Sandhu S. S. (1983). Clastogenetic and physiological response of chromosomes to nine pesticides in the *Vicia faba in vivo* root tip assay system. **Mutat. Res.** 124: 69 – 84.

Derscheid, L. A., Stanler, L. M. and Kratochnil, D. E. (1952). Differential response of barley varieties to 2, 4-Dichlorophenoxyacetic acid. **Agron. J.** 44: 182-188.

Deserres, F. J. (1978). Introduction: Utilization of higher plant systems as monitors of environmental mutagens. **Environ. Health Perspect.** 27: 3-7.

Devadas, N., Rajam, M. V. and Subash, K. (1986). Comparative mutagenicity of four organophosphorous insecticides in meiotic system of red pepper. **Cytologia** 51: 645-653.

Deysson, G. (1968) Antibiotic substances. In: International Review of Cytology. G. H. Bounes and J. F. Danielli (eds.). Acad. Press, New York. Pp.99-148.

Doxey, D. (1949). The effect of isopropylphenyl carbamate on mitosis in rye (*Secale cereale*) and onion (*Allium cepa*) **Ann. Bot.** 13: 329-336.

Dyer, K. F. and Hanna, P. J. (1970). Comparative mutagenic activity and toxicity of triethyl phosphate and dichlorvos in bacteria and *Drosophila.* **Mutat. Res.** 16: 175-177.

Dyer, K. F. and Hanna, P. J. (1972). Mutagenic and antifertility activity of trimethyl phosphate in *Drosophila melanogaster.* **Mutat. Res.** 21: 327-331.

Ehrenberg, L., Gustafsson, A. and Lundquists, V. (1961). Viable mutants induced in barley by ionizing radiations and chemical mutagens. **Hereditas** 47: 243-282.

Eigsti, O. J. and Dustin, P. Jr. (1957). Colchicine in Agriculture, Medicine, Biology and Chemistry. Lowa State College Press. Ames, U.S.A.

Eissa, R. A. and Eissa, A. A. (1990). Cytological effects of the insect growth regulator (IGR) Fenoxycarb on mitotic cells of onion (*Allium cepa*). **Annals of Agricultural Science, Moshtohor** 28: 2555-2565.

El-Khodary, S., Habib A. and Haliem, A. (1989a). Cytological effect of the herbicide Garlon-4 on root mitosis of *Allium cepa.* **Cytologia** 54: 37- 44.

El-Khodary, S., Habib, A. and Haliem, A. (1989b). Effects of the herbicide Goltix on root mitosis of *Allium cepa.* **Annals of Agricultural Science Cairo.** 34: 1103-1112.

El-Khodary, S. Habib, A. and Haleim, A. (1990). Effects of the herbicides Tribunil on root Mitosis of *Allium cepa.* **Cytologia** 55: 209-215.

Ellis, R. J. and Mac Donald, I. R. (1970) Does cycloheximide inhibits protein synthesis specifically in plant tissues? **Nature 22**: 791-792.

El-Sadek, L. M. (1972). Mitotic inhibition and chromosome aberrations induced by some arylasonic acids and its compounds in root tips of maize. **Egyptian Journal of Genetics and Cytology.** 1: 218-224.

El-Shazly, H. A. (1995). Genotoxicity of some mycotoxins and their effect on growth and yield of *Vicia faba*. Ph.D. thesis, Ain Shams University, Cairo, Egypt.

El-Shehaby, K. and Mohamed, H. A. (1985). Some side effects of fungicides on onion plants. **Agricultural Research Review** 63: 89-98.

Ene, E. E. and Emadi, O. C. (1987). Mitodepressive effects of water extracts of *Boerhaavia diffusa* and *Vernonia amygdalina* on *Allium cepa* root mitosis. **Cytologia** 52: 469-474.

Ennis, W. B. Jr. (1948). The cytological effect of O-isopropyl-N-phenyl-carbamate upon *Avena*. **Amer. Jour. Bot.** 35:15-21.

Epel, D. (1963). The effect of carbon monoxide inhibition on ATP level and the rate of mitosis in sea urchin egg. **J. Cell Biol.** 17: 315-319.

Epstein, S. S. and Legator, M. S. (1971). The Mutagenicity of Pesticides. Concepts and Evaluation. The MIT Press, Cambridge, England, p.220.

Epstein, S. S., Bass, W., Arnold, E. and Bishop, Y. (1970). Mutagenicity of trimethylphosphate in mice. **Science** 168: 584-586.

Evans, G. M., Ress, H., Smell, C. L. and Sun, S. (1972). The relationship between nuclear DNA amounts and the duration of the mitotic cycle. **Chromosomes Today** 3: 24-31.

Evans, H. J. (1977). Molecular mechanism in the induction of chromosome aberrations. In: Progress in Genetic Toxicology. Scott, D., Bridges, B.A. and Sobel, F. H. (eds.), Elseviser / North Holland Biomedical Press. Amsterdam.

Evans, H. J. and Savage, J. R. K. (1963). Relation between DNA synthesis and chromosome structure as resolved by X-ray damage. **J. Cell Biol.** 18: 525-540.

Evans, H. J. and Scott, D. (1964). Influence of DNA synthesis on the production of chromatid aberrations by X-ray and maleic hydrazide in *Vicia faba.* **Genetics** 49: 17-38.

Farwqui, S. A. (1961). Abnormal meiosis and polyad formation in *Heliotropium* and *Ophioglossum.* **Cytologia** 26: 182-187.

Fest, C. and Schmidt, K. J. (1973). The Chemistry of Organophosphorus Pesticides, Springer, Berlin.

Fishbein, L. (1972). Pesticidal, industrial food additive and drug mutagens. In: Mutagenic Effects of Environmental Contaminants. Sutton, H. L. and Harris, M. I. (eds.). Academic Press, New York, Pp.129-170.

Fishbein, L. (1976). Atmospheric mutagens. In: Chemical Mutagens, Principles and Methods for Their Detection. Hollander, A. (ed.), Plenum, New York Vol. IV, Pp. 219-319.

Flaks, J. C. and Cohen, S. S. (1959). Virus induced acquisition of metabolic function III. Formation and some properties of thymidylate synthetase of bacteriophage infected *Escherichia coli.* **Jour. Biol. Chem.** 234: 2981-2986.

Foray, L., Bertrand, C., Pingnet, F., Sonlier, M., Astre, C., Marion, C., Pelisser, Y. and Bessiere, J. M. (1999). *In vitro* cytotoxicity of three essential oils from *Salvia* species. **Journal of Essential Oil Research** 11: 522-526.

Ford, C. (1949). Chromosome breakage in nitrogen mustard treated *Vicia faba* root tip cells. **Proc. 8th Intern. Congr. Genet. Hereditas Suppl.** Pp. 570-571.

Freeze, E. and Bantz-Freeze, E. (1966). Mutagenic and inactivating DNA alterations. **Radiation Res.** 6: 97 – 140.

*Frey-Wyssling, A. (1938). Submikrostkopische Morphologic des Protoplasma und sciner Derivate. Born trager, Berlin.

Fujii, T. and Tadashi, I. (1983). Mutagenic effect of a pesticide (Ekatin) in the reference: soyabean (*Glycine max*) test system. **Environ. Exp. Bot**. 23: 97-102.

Gaur, B. K. and Beevers, H. (1959). Respiratory and associated responses of carrot discs to substituted phenols. **Plant Physiol**. 34: 427-432.

Gavrila, L. (1984). The stathmo – kinetic effect of Lindane: An unexpected property of a common insecticide in *Allium cepa* cytotoxicity. **Mutagenesis** 31: 35-36.

Gavrila, L., Langeanu, A., Rebedea, T., Ghetea, L., Gavrila, V. R. and Gavrila, L. B. (1994). The study of the mutagenic effects of zinc phosphide (a raticide) and of mitomycin C (an alkylating agent) in the presence of activated waters. **Cytologia** 59: 81-86.

Gelfant, S. (1963). Induction of cell division, a critical and experimental analysis. **Int. Rev. Cytol.** 14: 1-39.

George, N. M. and Ghareeb, A. (2001). Gene toxicity of the insecticide Cyolan on mitosis, meiosis and seed storage protein of *Vicia faba*. **Cytologia** 66: 77-84.

Ghareeb, A. and George, N. M. (1997). Cytotoxicity of insecticide Temik 15G (Decarb) in mitotic and meiotic cells of *Vicia faba* plant. **Cytologia** 62: 259-263.

Gichner, T., Velemnsky, J. and Pokorny, V. (1982). Somatic mutation induced by maleic hydrazide and its potassium and diethanolamine salts in *Tradescantia*. **Mutat. Res.** 103: 289-293.

Gimenz-Martin, G. and Lopez-Saez, J. F. (1961). The action of γ-hexachlorocyclohexane on cellular division. **Phyton.** 16: 45-55.

Giri, S., Sharma, G. P., Giri, A. and Prasad, S. B. (2002). Genotoxic effects of Malathion in chick *in vivo* micronucleus assay. **Cytologia** 67: 53-59.

Goetz, P., Sram, R. J. and Dalmalova, J. (1975). Relationship between experimental results in mammals and man. 1.Cytogenetic analysis of bone marrow injury induced by a single dose of cyclophosphamide. **Mutat. Res.** 31: 247-254.

Gomergen, A. N. (2000). Cytological effect of the herbicide 2, 4-D isooctylester 48% on root mitosis of *Allium cepa*. **Cytologia** 65: 383-388.

Gomes, J., Dawoder, A. H., Revitt, D. M and Anilal, S. V. (1999). Hepatic injury and disturbed amino acid metabolism in mice following prolonged exposure to organophosphorus pesticides. **Human and Experimental Toxicology.** 18: 35-37.

Govil, J. N. (1998). Current Concepts of Multidiscipline Approach to the Medicinal Plants. Part I Today and Tomorrow's Printers and Publishers, Karol Bagh, New Delhi. P. 22.

Graham, S., Dayal, S., Swanson, M., Mittleman, A. and Wilkinson, G. (1978). Diet in the epidemiology of cancer of the colon and rectum. **Journal of National Cancer Research Institute** 61: 709-714.

Grant, W. F. (1970). Pesticides and heredity. **Mac Donald J.** 31: 211-214.

Grant, W. F. (1971). The case for mutagenic testing of chemical pollutants. **Can. Field. Nat.** 85: 203-204.

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

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

Grant, W. F. (1994). The present status of higher plant bioassays for the detection of environmental mutagens. **Mutat. Res.** 310: 175-185.

Grant, W. F. and Elizabeth, T. O. (2001). Chromosome aberration assays in *Pisum* for the study of environmental mutagens. **Mutat. Res.** 488: 93-118.

Grover, I. S. (1988). Genotoxic effects of some organophosphorus pesticides. III. *In vivo* chromosomal aberration bioassay in root meristems of *Allium* and *Hordeum.* **Cytologia** 53: 181-191.

Grover, I. S. and Tyagi, P. S. (1979). Chromosomal aberrations induced by pesticides in meiotic cells of barley. **Caryologia** 33: 251-259.

*Grover, I. S., Dhingra, A. K., Adhikari, N. and Ladhar, S. S. (1990). **Progress in Clinical and Biological Research** 340: 91-106.

Grover, I. S., Ladhar, S. S. and Randhawa, S. K. (1989). Carbaryl-a selective genotoxicant. **Environmental Pollution** 58: 313-323.

Grundmann, E. (1966). General Cytology. Edward Arnold Ltd., London, P. 428.

Guha, S. and Das, B. K. (1994). Cytological effects of some pesticides on the plant *Allium cepa.* **Pesticide Research Journal** 6: 75-79.

Gumanov, G. D. and Gumanov, L. L. (1972). Mutagenic action of alkyl phosphates on T4B phage and alkylation phage DNA. **Dokl. Akad. Nauk. S.S.S.R.** 198: 1442-1444.

Gustavino, B., Vitagliano, E., Sothil, A. and Rozzoni, A. (1987). A comparison between short-term evaluation of micronuclei induced by x-ray and colchicine in root tips of *Vicia faba.* **Mutat. Res.** 192: 109-119.

Hada, Q., Bose, S. and Sinha, S. P. (1991). Vitamin C mediated minimisation of Malathion and Rogor induced mito-inhibition and clastogeny. **Cytologia** 56: 389-397.

Hada, Q. and Sinha, S. P. (1993). Vitamin C-mediated minimization of Rogor induced genotoxicity. **Mutat. Res. Genetic-Toxicology-Testing.** 299: 29-36.

Hadder, J. C. and Wilson, G. B. (1958). Cytological assay of C-mitotic and prophase poison action. **Chromosome** 4: 91-104.

Hakeen, H. and Amer, S. (1964). Effects of Podophyllin on the meiosis of *Vicia faba.* **Nat. Wiss.** 51: 416.

Haliem, A. S. (1990). Cytological effects of the herbicide Sencor on mitosis of *Allium cepa.* **Egyptian Journal of Botany.** 93: 93-104.

Hammouda, M. A., Amer, S. M. and Farah, O. R. (1966). The effect of 1-napthyl-N-methyl carbamate "Sevin" on germination, growth and yield of cotton plant. **Flora, Abt. A.** 157: 292-298.

Hanna, P. J. and Dyer, K. F. (1975). Mutagenicity of organophosphorus compounds in Bacteria and *Drosophila.* **Mutat. Res.** 28: 405- 420.

Hanaa, H., El-Shazly and El-Sheikh, I. A. (2000). Arrest of mitotic cycle and induction of chromosomal aberrations by aflatoxin B_2 in root cells of *Vicia faba* L. **Cytologia** 65: 113-122.

Hartley, D. and Kidd, H. (1983). The Agrochemicals Hand Book. Royal Society of Chemistry, Nottingham, England.

Hassall, A. K. (1982) Chemistry of Pesticides, Their Metabolism, Mode of Action and Uses in Crop Protection. Mac Millan Press, Hongkong. Pp. 67-69.

Heiner, R. F. (1971) Alteration in the nuclear cycle, mitotic index and chromosomes of *Vicia faba* as affected by diethyl sulphate. **Mutat. Res.** 12: 249-254.

Hepler, P. K. and Jackson, W. T. (1969). Isopropyl- N- phenyl carbamates affect the spindle microtubules orientation in dividing endosperm cells of *Haemanthus katherinae* Baker. **J. Cell Sci.** 5: 727-743.

Herich, R. (1969). The effect of zinc on the structure of chromosomes on mitosis. **Nucleus** 12: 81-85.

Herichova, A. (1973). Cytological study of the effect of isopropyl N- (3-chloro-phenyl) carbamate on the division and ultra structure of meristematic cells of barely (*Hordeum sativum* L.). **Physiologia Plantarum** 6: 85-107.

Hess, F. D. (1983) Mode of action of herbicide that affects the cell division. In: Pesticide Chemistry, Human Health Welfare and Environment. Miyamoto, J. and Kearney, P. C. (eds.), Pergamon Press, Ltd., Headington, Hill Hall Oxford, Pp. 420- 425.

Hidalgo, A., Gouzalez Reyes, J. A., Navas, P. and Garcia Herdugo, G. (1989). Abnormal mitosis and growth inhibition in *Allium cepa* roots induced by Propham and Chlorpropham. **Cytobios** 57: 7-14.

Hindmarch, M. M. (1951). A critical consideration of C-mitosis with reference to the effect of mitosis to nitrophenols. **Proc. Lin. Soc. N. S. Wales.** 76: 158-161.

*Hoffman – Berling, H. (1954). Die Bedentung des Adenosintriphosphates fur die zelkund Kernteilungshewegungen in der anaphase. **Biochem. Et. Biophys. Acta.** 15: 226-237.

Howard, A. and Pelc, S. R. (1953). Synthesis of deoxyribonucleic acid in normal and irradiated cells and its relation to chromosome breakage. In: Symposium on chromosome breakage. **Heredity 6. Suppl.** Pp. 261-273.

Hussein, F. and Hussein, M. G. (1963). Cytological effects of some organophosphorous compounds on *Vicia faba* L. **Jour. of Bot. UN Arab. Repub.** 6: 27-52.

Hutson, D. H. (1982). Formation of lipophilic conjugates of pesticides and other xenobiotic compounds. In: Progress in Pesticides Biochemistry. Hutson, D. H. and Roberts, T. R. (eds.), John Wiley, N. Y. Vol. II. Pp. 171-184.

Ignacimuthu, S. and Babu, C. R. (1988). Nuclear DNA content and RNA amounts in the wild and cultivated Urd and Mung beans and their M_1 plants. **Cytologia** 53: 535-541.

Ignacimuthu, S. and Kochuthressia, M. V. (1994). Effects of Monocrotophos on root tip cells of *Allium cepa* and *A. sativum*. **J. Cytol. Genet.** 29: 41-44.

Ignacimuthu, S. and Kumar, P. (1994). Effects of endosulfan on root tip cells of *Allium cepa*. **Journal of Ecotoxicology and Environmental Monitoring** 4: 211-215.

Imbamba, S. K. and Moss, D. N. (1971). Effect of Atrazine on physiological process in leaves. **Crop Sci.** 1: 844 - 848.

Jadhav, R. K. and Mungikar, A. M. (1998). Mitotic inhibition and chromosomal aberrations induced by deproteinized leaf juice of Lucerene (*Medicago sativa*, L.) in root tips on onion (*Allium cepa*). **J. Mendel** 15: 21-22.

Jain, A. K. and Bask, S. L. (1965). Experimental modification of chiasma distribution in *Delphinium*. **Indian J. Genet.** 25: 14-23.

Jain, A. K. and Sarbhoy, R. K. (1988). Cytogenetic studies on the effect of some chlorinated pesticides. **Cytologia** 53: 427-436.

James, D. and David, R. (1990). Magic and Medicine of Plants. The Reader's Digest Association, Inc, Pleasantville, New York.

Jansen, H., Muller, B. and Kwoblock, K. (1987). Allicin characterization and its determination by HPLC. **Plants Med.** 53: 559.

Jogoda, M. (1980). Cytological disturbances in *Allium cepa* L. root meristems induced by herbicides. **Acta Biologica-Cracoviensia-Botanica** 22: 189-211.

John, F. H., Jalal, S. M., Michael, J. E. and Martsolf, J. T. (1989). Genotoxicity of the organophosphorus insecticide Malathion on human lymphocytes in culture. **Cytologia** 54: 191-195.

Jones, H. A. and Mann, L. K. (1963). Onions and Their Allies. Inter-Science Public INC., New Delhi.

Kabarity, A. (1962). The effect of certain mutagenic substances upon prophases. **Beitr. Biol. Pflanzen** 42: 317-326.

Kabarity, A. and Mallalah, G. (1980). Mitodepressive effect of khat extract in meristematic region of *Allium cepa* root tips. **Cytologia** 45: 733-738.

Kabarity, A. and Nahas, A. (1979). Induction of polyploidy on C-tumours after treating *Allium cepa* root-tips with the herbicide "Treflan". **Biologia Plantarum (Praha)** 21: 253-258.

Kada, T. M., Moriya, K. and Shirasu, Y. (1974). Screening of pesticides for DNA interactions by re-assay and mutagen's testing and frame shift mutagens detected. **Mutat. Res.** 26: 243-248.

Kalia, C. S., Kharkual, M. C., Singh, M. P. and Alice, K. V. (2001). Mutagenic effects of environmental industrial chemical agents in inducing cytogenetical changes in wheat. **Indian J. Genet.** 61: 203-208.

Kamble, S. M., Mohekar, A. D., Bhagwan, H. K. and Kulkarni, D. A. (2002). Effect of sublethal concentration of carbamate on total protein and total free amino acids content in ovaries of fresh water fish, *Barilus bendelisis* and *Barilus burna*. **J. Aquatic Bio.** 17: 61-63.

Kandler, O. (1960). Energy transfer through phosphorylation mechanism in photosynthesis. **Annu. Rev. Plant Physiol.** 11: 37-54.

Kara, M., Sanda, M. A. and Ates, A. (1994). Cytogenetic effects of the insecticide Cypermethrin on the root meristems of *Allium cepa* L. **Turkish- Journal of Biology.** 18: 323-331.

Kato, Y. (1955). Responses of plant cells to gibberelline. **Bot. Gaz.** 117: 16-24.

Kaufman, B. P. (1956). Cytochemical studies of changes induced in cellular materials by ionizing radiations. **Ann. N. Y. Acad. Sci.** 59: 553.

Kaur, P. and Grover, I. S. (1985). Cytological effects of some organophosphorus pesticides 1. Mitotic effects. **Cytologia** 50: 187-197.

Kaur, T. and Saxena, P. K. (2001). Impact of pollution on the flesh of some fishes inhibiting river Satlej waters. A biochemical study. **Indian J. Environ. Health.** 44: 58-64.

Keer, R. W. (1963). Some recent advance in the study of insecticide resistance. **J. Australian Inst. Agric. Sci.** 29: 31 - 37.

Khandelwal, S. (1986). Effect of *Ophioglossum* oil on mitosis in *Allium sativum* and *Vicia faba*. **Cytologia** 51: 579-586.

Khynriam, D. and Prasad, S. B. (1999). Effect of Cisplatin on *Allium cepa* root meristem cells. **Cytobios** 100: 171-180.

Kihlman, B. A. (1956). Factors affecting the production of chromosome aberrations by chemicals. **J. Biophys. Biochem. Cytol.** 2: 543-555.

Kihlman, B. A. (1960). The radiomimetic effect of N-nitroso-N-methylurethan in *Vicia faba*. **Exptl. Cell Res.** 20: 657-659.

Kihlman, B. A. (1961). Biochemical aspects of chromosome breakage. **Advances in Genet.** 10: 1-5.

Kihlman, B. A. (1962). The production of chromatid aberrations by 5-fluorodeoxyuridine alone and in combination with X-rays and 8-ethoxy caffeine. **Caryologia** 15: 261-275.

Kihlman, B. A. (1966). Action of Chemicals on Dividing Cells. Prentice Hall Inc, New Jersey.

Kihlman, B. A. (1975). Root tips of *Vicia faba* for the study of the induction of chromosomal aberrations. **Mutat. Res.** 31: 401-412.

Kihlman, B. A. and Levan, A. (1949). The effect of purine derivatives on chromosomes. **Hereditas** 35: 393-396.

Kilbey, B. J., Legator M., Nicholas, W. and Ramel, C. (1977). Hand Book of Mutagenicity Test Procedures. Elsevier Scientific Publishing Co. Amsterdam. P. 485.

Klasterska, I., Natarajan, A. T. and Ramel, C. (1976). An interpretation of the origin of subchromatid aberrations and chromosome stickiness as a category of chromatid aberration. **Hereditas** 83: 153-169.

Klein, M. (1990). C-mitotic action of the insecticide Ambush 25 EC in *Allium cepa* L. **Genetica Polonica** 31: 107-113.

Kooffreh, M. (1999). Cytogenetic effects of glyphosate and 2:1 metolachlor: atrazine on root tips of *Allium cepa*. **Global Journal of Pure and Applied Science** 5: 317-332.

Kostoff, D. (1948a). A typical growth, abnormal mitosis, polyploidy and chromosome fragmentation induced by hexa chlorocyclohexane. **Nature** 162: 845-846.

Kostoff, D. (1948b). Cytogenetic changes and a typical growth induced by hexachlorocyclohexane ($C_6H_6Cl_6$). **Curr. Sci.** 17: 294-295.

Kozera, W. (1980). The influence of the fungicide Benlate and Topsin-M on the mitotic process in root meristems of onion sets. (*Allium cepa* L.) **Acta. Agarica - et. Silvestria-Agaria.** 19: 117-132.

Kumar, D., Khan, P. K. and Sinha, S. P. (1995). Cytogenetic toxicity and no effect limit dose of pesticides. **Food and Chemical Toxicology.** 33: 309-314.

Kumar, D. and Sinha, S. P. (1989). Threshold dose of cytogenetic toxicity of Lindane, Malathion and Metacid in *Allium cepa* root tip cells. **Cytologia** 54: 547-552.

Kumar, G. and Tripathi, A. (2003). Comparitive mitotoxicity and mutagenicity of two heavy metals in *Allium cepa*, L. **J. Cytol. Genet.** 4: 169-173.

Kumar, R. S., Chowdhury, R. and Mallick, R. (1989). Cytological effects of phosalone on root meristem of *Allium cepa* L. **Cytologia** 54: 429- 435.

Kumar, U. and Sinha, S. S. N. (1991). Genotoxic effects of two pesticides (Rogor and Bavistin) and antibiotic Streptomycin in meiotic cells of grass pea (*Lathyrus sativus* L). **Cytologia** 56: 209- 214.

Kumari, T. S. and Vaidyanath, K. (1989). Testing of genotoxic effects of 2, 4-dichlorophenoxy acetic acid (2, 4-D) using multiple genetic assay systems of plants. **Mutat. Res.** 226: 235-238.

Latt, S. A. (1975). Sister chromatid exchanges, indices of human chromosome damage and repair. Detection by fluorescence and induction by mitomycin C. **Proc. Natl. Acad. Sci. (U.S.A.),** 71: 3162-3166.

Lawley, P. D. (1966). Effects of some chemical mutagens and carcinogens on nucleic acids. **Program in Nucleic acids Research and Mol. Biol.** 5: 89-131.

Lawley, P. D. and Brooks, P. (1963). Further studies on the alkylation of nucleic acids and their constituent nucleotides. **Biochem. J.** 89: 137-138.

Lawley, P. D., Shah, S. H. and Orr, D. J. (1974). Methylation of nucleic acids by 2,2-dichlorovinyl dimethyl phosphates. (Dichlorvos, DDVP). **Chem-Biol. Interactions.** 8: 171-182.

Lazanyi, A. (1968). The effect of sulphoguanidine in CO-gamma irradiated G_{16} and G_2 cells on the incompleteness of exchange and the question of gaps in *Vicia faba* L. **Mutat. Res.** 6: 67-79.

Lea, D. E. (1962). Lethal Effects. In Action of Radiation on Living Cells. Cambridge University Press.

Lederberg, J. (1971). The Mutagenicity of Pesticides. The MIT Press, Cambridge.

Lee, Y. P. and Takahashi, T. (1966). An improved calorimetric determination of amino acids with the use of ninhydrin. **Anal. Biochem.** 14: 71 –77.

Leffler, H. R., O'Brien, T. J., Glover, D. V. and Cherry, J. H. (1971). Enhanced deoxyribonucleic acid polymerase activity of chromatin from soybean hypocotyls treated with 2, 4-dichloro-phenoxy acetic acid. **Plant. Physiol.** 48: 43-45.

Legator, M. S., Palmer, K.A. and Adler, I. D. (1973). A collaborative study of *in vivo* cytogenetic analysis. 1. Interpretation of slide preparation. **Toxicol. Appl. Pharmacol.** 24: 337-350.

Lehmen, L. P. Jr., Vaughan, M.A. and Vaughan, K. C. (1990). Terbutol affects spindle microtubule organizing centers. **Journal of Experimental Botany** 41: 537-546.

Levan, A. (1938). The effect of colchicine on root mitosis in *Allium*. **Hereditas** 24: 471-486.

Levan, A. (1949). Chemically induced chromosome reaction in *Allium cepa* and *Vicia faba*. **Cold Spring Harbor Symposium on Quantitative Biology.** 16: 238.

Levan, A. Tjio, J. H. (1948). Introduction of chromosome fragmentation by phenols **Hereditas** 34: 453-454.

Lewin, B. (1997). Genes VI. Oxford University Press, Oxford, Pp. 1089-1090.

Liang, G. H., Felter, K. C., Liang, Y. Y. S. and Morril, J. M. (1967). Cytogenetic effects and responses of agronomic characters on grain *Sorghum* following Atrazine application. **Crop. Sci.** 7: 245-248.

Liang, G., Feltner, K. C. and Russ, O. (1969). Meiotic and morphological response of grain *Sorghum* to Atrazine, 2, 4-D, oil and their combinations. **Weed Sci.** 17: 8-12.

Liao, S. H. and Hamlton, R. H. (1966). Intracellular localization of growth hormones in plants. **Science** 151: 822-824.

Lilley, L. J. (1965). Introduction of chromosome aberrations by aflatoxins. **Nature** 207: 433-434.

Lofroth, G. (1970). Alkylation of DNA by dichlorvos. **Naturwissenschaften** 57: 393-394.

Lofroth, G., Kim, C. and Hussein, S. (1969). Alkylating property of 2, 2-dichlorovinyl dimethyl phosphates and a disregarded hazard. **EMS Newslett.** 2: 21-27.

Lowry, O. H., Rosenbrough, N. J., Lewis Farr, A. and Randell, R. J. (1951). Protein measurement with folin phenol reagent. **J. Biol. Chem.** 19: 265 - 275.

Ma, T. H. (1982). *Vicia* cytogenetic tests for environmental mutagens. A report of the U.S. Environmental Protection Agency Gene Tox. Program. **Mutat. Res.** 99: 257 - 271.

*Maaloe, O. and Kjeldgaard N. C. (1966). Control of macromolecular synthesis. Benjamin, W.A. (eds.), New York, Amsterdam.

Mac Leode, R. D. (1969). Some effects of 2,4,5-trichlorophenoxy acetic acid on the mitotic cycle of lateral root apical meristems of *Vicia faba*. **Chromosoma** 27: 227 – 237.

Mandel, S. K. and Basu, R. K. (1981). Cytological effects of single and combined treatments with X-rays, IAA and GA_3 in *Allium cepa*. **Cytologia** 46: 133-139.

Mann, J. D. and Storey, W. B. (1966). Rapid action of carbamate herbicides upon plant cell nuclei. **Cytologia** 31: 203-207.

Mann, J. D., Young, K. H., Pu, M. and Haid, H. (1967). Phenylurethane herbicides. In: Inhibitors of Changes in Metabolic State. 1. Botanical Aspects. **Biochem. Biophys. Acta** 138: 133-139.

Mansour, K. S. (1984). Cytological effects of Tribunil on *Vicia faba* L. **Egypt. J. Bot.** 27: 191-198.

Markham, R. (1955). Nucleic acids, their components and related compounds. In: Modern Methods of Plant Analysis. Peach, K. and Tracy, M. V. (eds.), Springer –Verlag, Berlin. Vol. IV, Pp. 246 –301.

Marshall, W. K. and Roberts, J. R. (1978). Ecotoxicology of Chlorpyrifos. **National Research Council of Canada,** Ottawa.

Matsuoka, M., Hayashi, M. and Ishidater, Jr. (1979). Chromosomal aberration Tests on 29 chemicals combined with S_9 mix *in vitro*. **Mutat. Res.** 66: 277-290.

Matsuura, H. and Tanifunji, S. (1962). Chromosome studies on *Trillium kamtsghaticum*. **Biochem. Biophys. Acta** 247: 310-321.

Mattson, A. M., Spillane, J. T. and Pearce, G. W. (1955). Dimethyl 2,2 – dichlorovinyl phosphate (DDVP), an organic phosphate compound highly toxic to insects. **J. Agric. Food. Chem.** 3: 319.

Mazia, D. (1961). Mitosis and the physiology of cell division. In: The Cell. Brachet. J. and Mirsky, A. E. (eds.) III. Academic Press, NewYork. Pp.77- 412.

Mc Gill, M., Pathak, S. and Hsu, T. C. (1974). Effects of ethidium bromide on mitosis and chromosome. **Chromosoma**. 47: 157-167.

Mc Leish, J. (1953). The action of maleic hydrazide in *Vicia faba*. Symposium on chromosome breakage. **Heredity 6(suppl.):** 125-147.

Mc Manus, M. A. (1960). Certain mitotic effects of kinetin, gibberellic acid, indole acetic acid and maleic hydrazide on the root of *Allium cepa*. **Nature** 185: 44-45.

Meisner, H. M. and Sorensen, L. (1966). Metaphase arrest of Chinese hamster cells with rotenone. **Exp. Cell Res.** 42: 291-295.

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

*Metcalf, R. L. (1965). Research in Pesticides. Chichester C.O. (eds.), Springer-Verlag, Berlin, New York. Pp. 17 - 29.

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

Mitchison, J. M. (1977). The timing of cell cycles events. In: Mitosis Facts and Questions. Little, M., Poweltz, N., Petzett, C., Poustingle, M., Schroeder, D. and Zimmerman, H. P. (eds.), Springer-Verlag, New York, Pp. 14-19.

Mitchison, J. M. and Creanor, J. (1971). Further measurements of DNA synthesis and enzyme potential during the cell cycle of the fission yeast *Schizosaccharomyces pombe*. **Exp. Cell Res.** 69: 244-247.

Mohan, G. (1973). 5-Methyl tryptophan resistance mutations in *Escherichia coli* k_{12}. Mutagenic activity of monofunctional alkylating agents including organophosphorous insecticides. **Mutat. Res.** 20: 7-15.

Mohandas, T. and Grant, W. F. (1972). Cytogentic 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.

Momparler, R. L. and Myronkaron, S. E. S. (1976). Effect of adriamycin in DNA, RNA and protein synthesis in cell free systems and intact cells. **Cancer Res.** 36: 2981-2985.

Montesano, R., Bartsch, R. and Tomatis, L. (1976). Screening Tests in Chemical Carcinogenesis. IARC, Lyon.

Morgan, D. T. (1950). A cytogenetic study of inversion in *Zea mays*. **Genetics** 35: 153.

Morrison, I. M., Owino, M. G. and Stable, E. H. (1981). Effect of diclofap on growth, mitotic index and structure of wheat (*Triticum aestivum*) and wild oat (*Avena fatua*) adventitious roots. **Weed Sci.** 29: 426-432.

Morrison, J. W. (1962). Cytological effects of the herbicide Avadex. **Cand. J. Plant. Sci.** 42: 78-81.

Morse, D. E. and Yanofsky, C. (1969). Amber mutants of the trp R regulatory gene. **J. Mol. Biol.** 44: 185-193.

Mousa, M. (1982). Cytological studies on the effect of the herbicide Stomp on root tip cells of *Allium cepa*. **Egypt. J. Genet. Cytol.** 11: 15-22.

*Moustchen Dahmen, J. and Moutschen Dahmen, M. (1963). Interactions ioniques daus less effects radio mimetiques due methane sulfonate d'ethyl (EMS) surless chromosomes de *Vicia faba*. **Radiation Botany** 3: 297-310.

*Moutschen Dahmen, J. and Moutschen Dahmen, M. (1958). L'action du myleran (di-methane-sulfomyloxy-butane) Sur les chromosomes chez *Hordeum sativum* et chez *Vicia faba*. **Hereditas** 44: 415-446.

Muldoon, J. J., Evans, T. E., Nygaard O. F. and Evans, H. H. (1971). Control of DNA replication by protein synthesis at defined times during the S period in *Physarum polycephalum*. **Biochem. Biophys. Acta.** 247: 310-321.

Munro, H. N. and Fleck, A. (1966). The determination of nucleic acids. In: Methods of Biochemical Analysis. David Click (ed.), John Wiley & Sons, New York. Vol. XIV. Pp. 113 – 176.

Murthy, D. K. and Hanmanthu, P. (1985). Clastogenic and mitoclastic effects of benzimidazole derivatives. **Current Science** 54: 430-431.

Nagl, W. (1970). The mitotic and endomitotic nuclear cycle in *Artemisia carinatum* II. Relation between DNA replication and chromatin structure. **Caryologia** 23: 71-78.

Nagpal, A. and Grover I. S. (1994). Genotoxic evaluation of systemic pesticides in *Allium cepa* L. Mitotic effects. **The Nucleus** 37: 99-105.

Nagpal, A., Roni, G., Kaur, S. J., Arora, S. and Cheema, G. (2002). Genotoxic effect of two azo dyes in *Allium cepa*. **J. Cytol. Genet.** 3: 197-200.

Nainala, V. G. (1986). Effect of metaxuron (N (3-chloro, 4-methoxyphenyl) N, N dimethyl urea) on the meiotic behaviour in *Allium cepa* L. **Journal of the Indian Botanical Society** 65: 81.

Nanda, S. (1972). Nutritive value of Indian food. **Indian J. Med. Res.** 60: 1470.

Nandi, S. (1985). Studies on the cytogenetic effect of some mercuric fungicides. **Cytologia** 50: 921-926.

Narasinghani, V. G. and Kumar, S. (1976). Asynapsis and spindle abnormalities in EMS and MMS treated Peas (*Pisum sativum* L.) **Science and Culture** 42: 117-120.

Nasar, S. K. T. and Singh, S. (1980). Arrest of somatic cells at G_2 by Sevin (pesticide). **Experientia** 36: 948-949.

Natarajan, A. T. and Upadhya, M. D. (1964). Localized chromosome breakage induced by ethyl-methane sulfonate and hydroxylamine in *Vicia faba*. **Chromosoma** 15: 156-169.

Nelson, M. C., Jalal, S. M. and Larson, O. R. (1990). Genotoxicity of the organophosphorus insecticide Chloropyrifos based on human lymphocyte culture. **Cytologia** 55: 589- 592.

Nesy, E. A. (1991). Antimitotic and clastogenic properties of aluminium sulphate. M.phil Dissertation, University of Kerala, India.

Ockey, C. H. (1957). A quantitative comparison between the cytotoxic effects produced by proflavine, acetylethyleneimine and triethylene melamine on root-tips of *Vicia faba*. **J. Genet.** 55: 525-550.

Oksala, T. and Therman, E. (1974). Mitotic abnormalities and cancer. In: Chromosome and Cancer. German, J. (ed.). John Wiley and Sons, NewYork.

Oku, K. (1978). Chromosomal aberrations induced by maleic hydrazide under various pH conditions. **Chromosome Information-Service** 25: 8-9.

Oku, K. (1982). Effects of pre-treatment with AET on the chromosomal aberrations induced by MH in root tips of *Allium cepa* L. **Chromosome Information Service** 32: 11-12.

Olah, L. V. (1963). Effects of digitonin on cellular division. **Genetics Today** 1: 102-103.

Onfelt, A. and Klasterska, I. (1983). Spindle disturbances in mammalian cells and multiple chromatid exchanges after treatment of V_{79} Chinese hamster cells with Carbaryl. **Mutat. Res.** 19: 319-330.

Ostergen, B. A., Koopman, A. and Reitalu, J. (1953). The occurrence of the amphiastral type of mitosis in higher plants and the influence of aminopyrin on mitosis. **Bot. Notiser** 417- 419.

Panda, P. B. and Sahu, U. K. (1985). Induction of abnormal spindle function and cytokinesis inhibition in mitotic cells of *Allium cepa* by the organophosphate insecticide Fensulphothion. **Cytobios** 42: 147-155.

Panda, P. B. and Sharma, C. B. S. R. (1989). Organophosphate induced chlorophyll mutations in *Hordeum vulgare*. **Theor. Appl. Genet.** 55: 253 - 255.

Pandey, R. K., Shukla, R. and Datta, S. K. (1994). Chromotoxic effects of one fungicide (Dithane M-45) and two insecticides (Aldrex-30 and Metacid-50) **Cytologia** 59: 419-422.

Pandita, T. K. (1986). Mutagenic studies on the insecticide Metasystox-R with *Allium cepa*. **Cytologia** 51: 387-392.

Pandita, T. K. and Khoshoo, T. N. (1984). Mutagenicity testing of Thimet 10-C. **The Nucleus** 24: 147-161.

Pandita, T. K., Pal, M. and Khoshoo, T. N. (1981). Cytological effects of Bavistin. **The Nucleus** 24: 106-111.

Papes, D., Besendorfer, V. and Bosiljevac, V. (1989). The *Allium* test response to cyanazine. **Acta Botanica Croatica** 48: 39-46.

Patil, B. C. and Bhat, G. I. (1992). A comparative study of MH and EMS in the induction of chromosome aberration on root meristem of *Clitoria ternatia* L. **Cytologia** 57: 259-264.

Patil, T. M. and Shirashyad, V. S. (1991). Effect of Methylparathion and Phosphamidon on mitotic chromosomal aberrations in some vegetable seeds. **Journal of Environmental Biology.** 12: 249-254.

Patnaik, S., Saran, B. K. and Patnaik, S. N. (1984). Effect of Zarda (Processed tobacco leaf) extract on the chromosome of *Allium cepa* L. **Cytologia** 49: 807-814.

Peter, F. (1993). Genetic toxicity of Malathion, A review. **Environmental and Molecular Mutagenesis.** 22: 717.

Pickett-Heaps, J. D. (1967). Further observation on golgi apparatus and its function in the cells of wheat seedlings. **J. Ultr. Res.** 18: 287-303.

Prakash, N. S. N. and Lakshmi, I. I. (1988). Cytological effects of agricultural chemicals II. Effects of fungicide Bavistin and Deltan on *Capsicum annum*. **Cytologia** 53: 709-815.

Prasad, A. B. (1974). Production of multipolar spindles in canary grass following irradiation of dry seeds. **J.Cytol.Genet**. 788: 184-187.

Prasad, G. and Das, K. (1977). Effects of some growth substances on mitosis. **Cytologia** 42: 323-329.

Prasad, I. and Pramer, D. (1968). Cytogenetic effects of Propanil and its degradation products on *Allium cepa*. **Cytologia** 34: 351-352.

Praveen, M. and Santhosh, K. (2001) Effect of dichlorvos on acetylcholine esterase in brain of rats. **Indian J. Environ. Toxico.** 11: 75-77.

*Preussmann, R., Schneider, H. and Epple, F. (1969). Untersuchungen and Nachweisalkylierender Agentien II. Der Nachwei's Verschiedener Klasen alkylierender Agentien mitciner modification der Farbreaktion mit 4-(4Nitrobenzyl)-pyridin, **Arzneinuttelfors Chung** 19: 1059-1073.

Pritchard, A. J. and Court, R. D. (1966). The cytological effects of mimosine. **Cytologia** 33: 73-77.

Priya, E. J., Vivekanandan, O. S. and Gowrishankar, G. (1996). Genotoxic effect of an organophosphorous pesticide on *Allium* root meristems *in vivo*. **Indian Journal of Experimental Biology** 34: 320-324.

Pushpangadan, P., Sobti, S. N., Khosala, S. N., Rao, B. L. and Khosla, M. K. (1979). Cytopathic and chromosomal effects of Parthenin on plant cells. **The Nucleus** 22: 146-148.

Rabindra, K., Rupak, C. and Ranajit, M. (1989). Cytological effects of Phosalone on root meristem of *Allium cepa*. **Cytologia.** 54: 429-435.

Raghuvanshi, S. S. and Singh, A. K. (1976). Effects of gamma rays on growth and karyokinetic activity in *Trigonella-foenum-graceum* L. **Cytologia** 41: 177-186.

Rank, J., Jenson, A. G., Skov, B., Fe Dersen, L. H. and Jenson, K. (1993). Genotoxicity testing of the herbicide Round up and its active ingredient

glyphosate isopropylamine using the mouse bone marrow micronucleus test, *Salmonella* mutagenicity test, and *Allium* anaphase-telophase test. **Mutation Research Genetic Toxicology Testing** 300: 29-36.

Rao, B. V. (1987). Effect of two agrochemicals (Didicofol and Paraquat) on mitotic divisions in *Allium cepa* root meristems. **Indian Journal of Botany** 10: 192-198.

Rao, B. V., Rao, B. G. S. and Sharma, C. B. S. R. (1988). Cytological effects of herbicides and insecticides on *Allium cepa* root meristems. **Cytologia** 53: 255-261.

Rao, B. V., Sharma, C. B. S. R. and Rao, B. G. S. (1986). Clastogenic and turbogenic effects of organophosphorus pesticides on *Allium cepa* root meristems. **Proceedings of the Indian Academy of Sciences, Plant Sciences** 96: 303-309.

Rao, B. V., Sharma, C. B. S. R. and Rao, B. G. S. (1987). Cytological effects of organophosphorus insecticides on *Allium cepa* root meristems. **Cytologia** 52: 365-371.

Rao, S. and Rao, S. (1986). Chromosomal aberrations induced by pesticides Endosulfan and Rogor in mitotic cells of *Allium cepa* L. **Journal of the Indian Botanical Society** 65: 80-81.

Ravindran, P. N. (1970). Cytological effects of Folidol. **Cytologia** 36: 504-508.

Ray, M. and Barman, A. (1987). Effect of industrial waste on root meristematic cells of *Allium sativum* L. **Phytomorphology** 37: 359-364.

Reddi, T. V. V. S. and Reddi, V. R. (1985). Cytological effects of chemical mutagens in rice. **Cytologia** 50: 499-505.

Reddy, M. V. and Rao, B. V. (1968). The cytological effects of insecticides (Dimecron -100 and Rogor-40) on *Vicia faba*. **Cytologia** 34: 408-417.

Reddy, S. S. and Rao, G. M. (1981). Cytogenetic effects of agricultural chemicals. 1. Effects of insecticides 'BHC' and 'Nuvacron' on chromosomes in chilli (*Capsicum annum L).* **Cytologia** 46: 699 - 707.

Reddy, S. S. and Rao, G. M. (1982). Cytogenetic effects of agricultural chemicals. II. Effects of herbicides Lasso and Basagram on chromosomal mechanism in relation to yield and yield components in chilli (*Capsicum annum* L.). **Cytologia** 47: 257-267.

Redei, G. P. (1998). Genetics Mannual. Current Theory, Concepts and Terms. World Scientific Publishing Co. Pvt. Ltd. Singapore.

Reider, C. L. (1982). The formation, structure and composition of the mammalian kinetochore and kinetochore fibre. **Int. Rev. Cytol.** 79: 1-58.

*Reiger, R. and Michaelis, A. (1960a). Chromatiden aberrationen nach Einwidkung Von Athyl methansulfonate auf Primarwurzeln Von *Vicia faba,* L. **Die Kulturpflanze (Berlin).** 8: 243.

*Reiger, R. and Michaelis, A. (1960b). Uber die radiomemetische Wirkung Von Athylalkohal bei *Vicia faba*. Chemische Mutagenese. Erwin-Baur-Gedam Chtnisvorlesungen 1. **Deutschen Akademic der Wissenschaften Zu Berlin.** Pp. 54-65.

Reiger, R., Michaelis, A. and Green, M. M. (1976). A Glossary of Genetics and Cytogenetics. Springer Verlag, Berlin.

Revell, S. H. (1953). Chromosome breakage by X-rays and radiomimetic substances in *Vicia faba*. **Heredity** 6 (Suppl.): 107-124.

Rosenkranz, H. S. and Rosenkranz, S. (1972). Reaction of DNA with phosphoric acid esters, gasoline additives and insecticides. **Experientia** 28: 386-387.

Rost, T. L. (1977). Responses of the plant cell cycle to stress. In: Mechanisms and Control of Cell Division. Rost, T. L. and Gifford, E. M. (eds.), Dowden Hutchinson and Ross Stroudsburg, Pp. 111-143.

Rost, T. L. and Reynold, T. (1985). Reversal of chlorosulfuron induced inhibition of mitotic entry of isoleusine and valine. **Plant Physiol.** 77: 481-482.

Ruan, C., Lian, Y. and Linn, J. (1992). Application of micronucleus test in *Vicia faba* root tips in the rapid detection of mutagenic environmental pollutants. **Chineese J. Environ. Sci.** 4: 56-58.

Rubin, B. A. and Methinsky, S. (1958). A study of the action of ionizing reactions on the metabolism of potato tubers in relation to their problem of all year round stage. In. **Proce. Int. Conf. Peaceful Use of Atomic Energy. Geneva** 27: 437-443.

Rudd, R. L. (1964). Pesticides and the Living Landscape. University of Wisconsin Press, Madison, Wisconsin.

Sabale, A. B. and Mane, A. A. (2000). Cytotoxic effects of maleic hydrazide on mitosis in *Allium cepa* varieties. **Advances in Plant Sciences.** 13: 179-184.

Sadhu, S. S. and Walters, M. D. (1980). Mutagenicity evaluation of chemical pesticides. **J. Environ. Sci. Health.** 15: 929-948.

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

Sakamoto, E. T. and Takahashi, C. S. (1979). Effects of the fungicide Dithane M-45, Benlate and Vitavax75 on the mitotic indices of *Allium cepa* root meristems. **Ciencia-e-Cultura.** 31(Suplemento): 573.

Sakari, K., Martti, S. and Pekka, V. (1981). Chromosome pulverization in blood diseases. **Hereditas** 95: 15-24.

Saliem A. R., Hussein, M. M., Allam, H. Z. and Farah, A. R. (1981). The effect of three synthetic organic insecticides on cytological characters in cottons. **Bull. Foe. Agric. Cairo University** 32: 52-66.

Samad, A., Guha, S., Misra, S. and Das, B. K. (1992). Cytological effects of Endosulfan (Thiodan) on root-mitosis of *Allium cepa* and *Vicia faba*. **Journal of Bengal Natural History Society** 11: 72-81.

Sato, S. G. and Tanaka, R. (1972). Effects of gallic acid derivatives on mitotic cells. **La kromosoma** 88 & 89: 2718.

Sattar, N. and Vahidy, A. A. (1988). Genotoxic effects of pesticides Endrin and Benlate. **Genome** 30**(Supplement)** 1: 324.

Sax, K. (1940). An analysis of X-ray induced chromosomal aberrations in *Tradescantia*. **Genetics** 25: 41-65.

Sax, K. (1960). Meiosis in interspecific pine hybrids. **Forest Sci.** 5: 135-138.

Schmidt, G. and Thannhauser, S. J. (1945). A method for the determination of deoxyribonucleic acid, ribonucleic acid and phosphoprotein in animal tissues. **J. Biol. Chem.** 161: 83 – 89.

Schneiderman, M. H., Dewey, W. C. and Highfield, D. P. (1971). Inhibition of DNA synthesis in synchronized Chinese hamster cell treated in G_1 with cycloheximide. **Exp. Cell Res.** 67: 147-155.

Schultz, D. and Funderburk, H. (1967). Effect of the herbicide Trifluralin on the nucleic acids of corn seedlings. **Plant Physiol.** 42: 50-53.

Scott, D. (1968). The additive effect of X-rays and maleic hydrazide in inducing chromosomal aberrations at different stages of the mitotic cycle in *Vicia faba*. **Mutat. Res.** 5: 65-92.

Scott, D. and Zampetti-Bosseler (1980). The relationship between cell killing, chromosome aberrations, spindle defects and mitotic delay in mouse

lymphoma cells of differential sensitivity to X-rays. **Int. J. Radiat. Biol.** 37: 33-47.

Shamberger, R. J. (1984). Genetic toxicology of ascorbic acid. **Mutat. Res.** 133: 135-159.

Sharda, S., Rao S. R. V. and Shah, V. C. (1973). Cytological and autoradiographic studies of the effects of vincaleukoblastine on the root meristem cells of *Vicia faba.* **Cytologia** 38: 1-9.

Sharma, A. K. and Chaudhuri, M. (1959). Gammexane (Hexa-chloro-cyclohexane) effect on chromosomes. **Curr. Sci.** 28: 498-499.

Sharma, A. K. and Gautam, D. C. (1991). Chromosomal aberrations induced by Phosphamidon and Endosulphan in the bone marrow cells of mice *in vivo.* **Cytologia** 56: 73-78.

Sharma, A. K. and Sharma A. (1960). Spontaneous and chemically induced chromosome breaks. **Int. Rev. Cytol.** 19: 101 – 136.

Sharma, A. K. and Sharma, A. (1990) Chromosome Techniques. Theory & Practice 3rd (ed.), Butter Worths, London.

Sharma, M (1980). Effect of the precedence of methyl, methoxy and hydroxyl groups in chemical compounds on chromosomes. **Indian Journal of Experimental Biology.** 18: 786-790.

Shehab, A. S. (1979). Cytological effect of medicinal plants in Quatar. 1. Mitotic effects of water extracts of *Pulcaria crispa* on *Allium cepa.* **Cytologia** 44: 607-613.

Shehab, A. S. (1985). Cytological effects of *Vinca* alkaloid on meiosis of *Vicia faba* **Cytologia** 50: 427-431.

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.

Sieler, J. P. (1978). Herbicidal phenylalkylureas as possible mutagens. II. Chemical basis for mutagenic activity. **Pestic. Biochem. Physiol.** 12: 183-190.

Simon, A. (1987). Cytological and morphological effects induced by physical and chemical mutagens in sunflower (*Helianthus annus* L.) Ph.D. Thesis Kerala University, Trivandrum, India.

Singh, M. R. (1982). Effects of IAA with MH and colchicine on root tip mitosis. **Cytologia** 47: 419-426.

Sinha, R. K., Choudhury, R. and Mallick, R. (1989). Cytological effects of Phosalone on root meristem of *Allium cepa* L. **Cytologia** 54: 429-435.

Sinha, S. P. (1988). Genotoxicity of pesticides. In: Perspectives in Cytology and Genetics. G. K. Manna (ed.), New Delhi, Vol. VI.

Smith, H. H. and Srb, A. (1951). Induction of mutation with β-propiolactone. **Science** 11: 490-492.

Smith, L. (1951). Cytology and genetics of barley. **Bot. Rev.** 17: 134-202.

Sobhi, H. M. and Haliem, A. S. (1990). Effects of herbicide Rancho (Mefenacet) on root mitosis of *Allium cepa* (onions). **Egyptian Journal of Botany.** 33: 43-50.

Sofradzija, A., Hadziselimovic, R. and Zovko, D. (1988). Mutagenic and genotoxic effects of some herbicides in meristem tissue of *Allium cepa*. **Genetika-Vugoslavia** 20: 183-189.

Soh, W. Y. and Yang, W. Y. (1993). Effect of plant growth regulators on mitotic chromosomes in *Allium cepa* L. **The Nucleus** 36: 109-113.

Somasekhar, R. K. (1984). Mitodepressive and clastogenic effects of fungicide Benlate in the root meristem of *Allium cepa*. **Egyptian Journal of Genetics and Cytology** 13: 207-213.

Somasekhar, R. K. (1988). Mitodepressive and clastogenic effects of the fungicide Brassicol in root meristem of *Allium cepa*. **Journal of the Indian Botanical Society.** 67: 223-224.

Somasekhar, R. K. and Arckal, G. D. (1983). Chromosomal aberrations induced by electroplating wastewater. **Cytologia** 48: 621-625.

Somasekhar, R. K. and Gouda, M. T. G (1984). Effect of fungicide Vitavax on *Allium cepa*. **Cytologia** 49: 177-182.

Somasekhar, R. K., Gowda, M. T. G. and Venkata Subaih, P. (1984). Cytological effects of fungicide Topsin in *Allium cepa*. **Cytologia** 49: 171-176.

Somasekhar, R. K., Venkata Subaih, P. and Gowda, M. T. G. (1987). Cytological effects of a fungicide Rizolex in *Allium cepa* L. **Pesticides** 21: 21-23.

Sparrow, A. H. (1961). Cytogenetic effects of ionizing radiations. In: Symposium on Mutation and Plant Breeding. NAC-NRC. Washington D. C., P. 891.

Sparrow, A. H. and Singleton, W. R. (1953). The use of radio cobalt as a source of gamma rays and some effects of chronic irradiation on growing plant. **Amer. Nat.** 87: 29 - 48.

Sreedevi, S. and Bindu, S. (2004). Cytotoxicity of alluminium sulphate on root tip cells of *Allium cepa* L. **J. Cytol. Genet.** 5: 77- 82.

Steffensen, D. (1953). Induction of chromosome breakage at meiosis by magnesium deficiency in *Tradescantia*. **Proc. Natl. Acad. Sci.** 39: 613-620.

Steffensen, D. (1955). Chromosome breakage with a calcium deficiency in *Tradescantia*. **Proc. Natl. Acad. Sci**. 41: 155-160.

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

Stevens, R. W. (1971). Pesticides in the Environment. Marcel Dekker Inc. New York, Vol. I, Part II, Pp. 310-399.

Stockert, J. C., Colman, O. D., Rimen, M. C. and Fernandes-Gomez, E.M. (1971). Nucleolar segregation by cold 2,4-dinitrophenol or anoxia in plant cells. **Cytologia** 36: 499-504.

Sudhakar, R., Ninge Gouda, K. N. and Venu, G. (2001). Mitotic abnormalies induced by silk dying industry effluents. **Cytologia** 66: 236-239.

Sudhakaran, K., Britto, A. J. and De Brittol, A. J. (1994). Effect of Metacid-50 on mitosis of *Allium cepa*. **Journal of Ecotoxicology and Environmental monitoring** 4: 101-104.

Sugii, M., Suzuki, T., Kakimoto, T. and Kato, Y. (1964). Sulfur containing amino acids and related compounds in garlic. 1. Assimilation of sulfates in garlic. **J. Bull. Ind.Chem. Res. Kyoto University.** 42: 246.

Suneson, C. A. and Jones, L. G. (1960). Herbicides may produce instability. **Agron. J.** 52: 120-121.

Surendra Nath, B., Sathyanarayana Raju, C. L., Radha Krishna, P.G. and Surendra Kumar, R. P. (1996). Effect of organophosphorus insecticides on protein and nucleic acids content of fat body and silk gland of the silk worm, *Bombyx mori*. **J. Environ. Bio.** 17: 269-272.

Sushama K., Laware, S. L. and Dhumal, K. N. (1999). Effect of Ultragin and Tribhuvan kirti on root tip cells of *Allium cepa* L. **J. Cytol. Genet.** 34: 147-151.

Swann, M. M. (1954). The control of cell division. In: Recent Developments in Cell Physiology. J. A. Kitching (ed.), Butterworths, London.

Swanson, C. P. and Merz, T. (1959). Factors influencing the effect of β-propiolactone on chromosomes of *Vicia faba*. **Science** 129: 1364-1365.

Swietlinska, Z., Zaborouska, D. and Zuk, J. (1973). Induction of chromosome aberration by diepoxybutane and caffeine in root meristems and germinating seeds of *Vicia faba*. **Mutat. Res.** 17: 199-205.

Swietlenska, Z., Zaborouska, D. and Zuk, J. (1974). Effect of cycloheximide on protein and DNA synthesis and on the yields of chromosomal aberrations induced by DEB and ENU in *Vicia faba*. **Mutat. Res.** 22: 33-38.

Takatori, S. (1965). Effects of some respiratory inhibitors on the duration of the different stages of the mitotic cycle of *Vicia* root tip cells. **Cytologia** 31: 220-235.

Taylor, E. W. (1973). Macromolecular assembly inhibitors and their action on the cell cycle. In: Drugs and the Cell Cycle. Zimmerman A. M., Padilla G. M. and Cameron I. L. (eds.), Academic Press, New York. Pp. 12-13.

Taylor, J. H., Hant, W. F. and Tung, J. (1962). Effects of fluorodeoxyuridine on DNA replication, chromosome breakage and reunion. **Proc. Natl. Acad. Sci. U.S.** 48: 190-198.

Therman, E. and Timomen, E. (1950). The prophase index and the occurrence of multiploar division in human cancer cells. **Hereditas** 40: 313-324.

Tomkins, D. J. and Grant, W. F. (1972). Comparative cytological effects of the pesticides Menazon, Metobromuron and tetra chloro isophthaponitrile in *Hordeum* and *Tradescantia*. **Can. J. Genet. Cytol.** 14: 245 – 256.

UNEP (1975) The International Register of Potentially Toxic Chemicals. (IRPTC). UNEP Brochure, September.

Unraw, J. and Larter, E. N. (1952). Cytogenetical response of cereals to 2, 4-D. 1. A study of meiosis of plant treated at various stages of growth. **Can. J. Bot.** 30: 22-27.

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

Van Huystee, R., Jachymazyk, B., Tester, C. F. and Cherry, J. H. (1968). X-ray irradiation effects on protein synthesis and synthesis of RNA from peanut cotyledons. **J. Biol. Chem.** 243: 2315-2320.

Vant' Hoff, J. (1968). The action of IAA and kinetin on the mitotic cycle of proliferative and stationary phase excised root meristems. **Exp. Cell Res.** 51: 167-176.

Vant' Hoff, J. and Wilson, G. B. (1962). Studies on the control of mitotic activity. The effect of respiratory inhibitors on mitotic cycle times in the root meristems of *Pisum sativum*. **Chromosoma** 13: 39-46.

Varner, J. E. (1964). Gibberellic acid controlled synthesis of ∝-amylase in barley endosperm. **Plant Physiol.** 39: 413-415.

Vaughan, M. A. and Vaughan, K. C. (1987). Pronamide disrupts mitosis in a unique manner. **Pesticide Biochemistry and Physiology** 28: 182-193.

Veleminsky, J. and Gichner, T. (1963). Cytological and genetic effects of the insecticide Systox on *Vicia faba* L. and *Arabidopsis thaliana* L. (Heynh) **Biol. Plant.** 5: 41-52.

Venkatarajan, S. (1981). Meiotic and breeding behaviour of EMS induced multilocular in *Capsicum annum* L. **J. Indian Bot. Soc.** 62: 133-137.

Vig, B. K. (1971). Increase induced by colchicine in the incidence of somatic crossing over in *Glycine max*. **Theor. Appli. Genet.** 41: 145-149.

Voogd, C. E., Jacobs, J. J. J. A. A. and Van Der Stel J. J. (1972). On the mutagenic action of dichlorvos. **Mutat. Res.** 16: 413-416.

Wacchter, D. E. and Baserga, R. (1982). Genes and regulation of the cell cycle in genetic expression in the cell cycle. **Academic Press. Inc.** 23: 244.

Wain Wright, M. (1978). A review of the effects of pesticides on microbial activity in soils. **J. Soil Sci.** 29: 287 - 298.

Wali, R. K., Singh, R., Dudeja, P. R., Sarkar, A. J. and Mohmood, A. (1984). Intestinal problem in test animals exposed to Malathion. **Bulletin of Environmental Contamination Toxicology.** 33: 289-294.

Walter, Z., Czajokouski, A. and Lipeck, K. (1980). Effect of Malathion on genetic material of human lymphocytes stimulated by phyto-chem-agglutin. **Hum. Genet** 53: 375-381.

Webster, P. L. and Davidson, D. (1969). Changes in the duration of the mitotic cycle induced by colchicine and indol-3yl acetic acid in *Vicia faba* roots. **J. Exp. Bot.** 20: 671-685.

Weinbach, E. C. (1956). Biochemical basis for the toxicity of pentachlorophenol. **Science** 124: 940.

Wild, D. (1973). Chemical induction of streptomycine resistant mutation in *Escherichia coli*. Dose and mutagenic effects of dichlorvos and methyl methane sulphonate. **Mutat. Res.** 19: 33-41.

Wild, D. (1975). Mutagenicity studies on organophosphorus insecticide. **Mutat. Res.** 32: 133 - 150.

Wilson C. L., Solar, J. M., Ghaouth, A. E. and Wismiewski, M. E. (1997). Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea.* **Plant Disease** 81: 204-210.

Wolff, S. (1959). Interpretation of induced chromosome breakage and rejoining. **Rad. Res. (suppl.)** 1: 453-462.

Wolff, S. and Luippold, H. E. (1955). Metabolism and chromosome-break rejoining. **Science** 122: 231-232.

Wooder, M. F. and Wright, A. S. (1981). Alkylation of DNA by organophosphorus pesticides. **Acta Parmacol. Toxicol.** 49: 51-55.

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

Wuu, K. D. and Grant, W. F. (1966b). Morphological and somatic chromosomal aberrations induced by pesticides in barley (*Hordeum vulgare*). **Can. J. Genet. Cytol.** 8: 481- 501.

Wuu, K. D. and Grant, W. F. (1967a). Chromosomal aberrations induced in somatic cells of *Vicia faba* by pesticides. **Nucleus** 10: 37-46.

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

Yakar, N. (1952). Meiotic disturbances caused by Chloranil (fungicide). **Amer. J. Bot.** 39: 540-546.

Yang, W., Yang, X. and Xia, Y. (1997) Active anticarcinogenic chemical components in ordinary garlic and selenium enriched garlic. **Chung Hua Yu Fang, 1. Hsuch Tsa Chich.** 31: 304- 306.

Yearly, L. P. and Stone, B. F. (1975). The effect of ionizing radiation and ribosomal RNA synthesis in grant rapid lettuce seeds. **Radiat. Bot.** 15: 153-159.

Yonus, S. E. A., Abdous, R. F. and Sherif, T. H. I. (1988). The effect of Nuvacron on the mitotic behaviour of *Vicia faba* L. **Cytologia** 53: 227-231.

Yoshida, Y., Nakamura K. J. and Hiura A. (1983). Contraction of chromosomes and depression of RNA synthesis by isopropyl N – (chlorophenyl) carbamate (CPC) in *Vicia faba* root cells. **Cytologia** 48: 707 – 717.

*Younis, S. A., Al-Hakkak, Z. S., Al-Rawi, F. I. and Hagop, E. G. (1989). **Journal of Stored Products Research** 25: 25-30.

Yow, W. C., Bolt, W. J., Chang, Y. S., Ershow, A. G., Yang, Z. T., An, Q., Handerson, B., Xu, G. W., Fraumen, J. F. Jr. and Wang, T.G. (1988). Diet and high risk of stomach cancer in Shandong. **China Cancer Research** 48: 3518 - 3523.

Zaheer, S. H. (1948). The Wealth of India VII. Publication and Information Directorate CSIR, New Delhi.

Zart, F., Heinz, E., Fernandes, M. I. B., De, M. and Michelin, E. (1979). Cytological effects of crop protection chemicals on onion (*Allium cepa*) root tips. **Ceincia-e-Cultura** 31: (Suplemento) 575.

Zirkle R. E. (1970). Ultra violet microbeam irradiation of newt-cell cytoplasm, spindle destruction, false anaphase and delay of true anaphase. **Radiat. Res.** 41: 516-537.

Zuk, J. and Swietlinska, Z. (1973). Effect of caffeine on nucleic acid and protein synthesis in V*icia faba* **Mutat. Res.** 17: 207-212.

*Original not referred