REVIEW OF LITERATURE

1. CHEMICAL TREATMENT AND CELL DIVISION

Mitosis and meiosis are fundamental activities of living cells required for the growth and differentiation of an organism. These two basic processes of cell division may be inhibited by certain chemical compounds called as 'Mitotic poisons' (Biesele, 1958). These chemicals may be endogenous or exogenous and may inhibit different phases or all phases of mitosis with different intensity. Mitotic mechanisms such as nuclear membrane cycle, chromosomal condensation, behaviour of the centromere, formation of spindle, chromosome duplication, mechanism of nuclear DNA, cytoplasmic RNA and protein etc. may be affected by these agents. Different types of mitotic poisons and their effect on cell division are briefly summarized here.

i) Mitotic poisons acting at prophase and interphase

Some chemicals inhibit oxidation or uncouple oxidative phosphorylation process which provide energy for mitosis. These chemicals prevent mitosis and not chromosomal duplication. Cyanide, azide, 2-4 dinitrophenols are common examples. A few chemicals interfere with chromosomal replication by affecting DNA and protein synthesis. Chromosomal fragmentation or other aberrations are produced by some agents such as ionizing radiations. When the chemical agent is highly active it brings about nuclear disintegration and genetic material flows out of the nucleus

resulting in nuclear vacuolation and lysis (Saez and Drets, 1958).

ii) Mitotic poisons acting at metaphase and other phases

This type of action is known as C-mitosis because it is produced principally by colchicine (Dustin *et al.*, 1937). Colchicine is an alkaloid which affects the formation of spindle apparatus and chromosomal division in metaphase and anaphase to different degrees. Chromosomal duplication is not affected hence polyploidy may occur. Colchicine binds to tubulin (basic protein unit of microtubules) preventing its polymerization and the formation of microtubules and thus blocks the spindle formation.

2. CYTOLOGICAL STUDIES IN *ALLIUM CEPA*

The karyotype of *Allium cepa* has been described by Mensinkai (1939). The complement consists of eight pairs of chromosomes (2n = 16) as follows: Five pairs of chromosomes with centromeres situated median to submedian, two pairs in which the centromeres are submedian and one pair of satellite chromosomes in which the satellites are situated at the end of the short arm. The size of chromosome varies from 8 to 16 μm depending upon the preperation. The duration of mitotic cycle in *Allium cepa* depends upon the temperature under which the experiments are carried out. Several authors

have determined this time period which varied from 13.5 to 23 hours in different studies (Lopez - Saez *et al.*, 1966; NutiRonchi and Arcara, 1967; Gonzalez - Fernandez *et al.*, 1966; Matagne, 1968).

According to Grant (1982) the common onion is an excellent plant for the assay of chromosome aberrations after chemical treatment. A classical test for studying the effect of chemicals on plant chromosomes was developed by Levan (1938, 1949) using root tips from bulbs of *Allium cepa*. It is known as *Allium test*. Grant (1982) has given a detail of protocols for using root tips from either bulbs or seeds of onion to study the cytological end points such as chromosome breaks and exchanges. A survey of chemicals used in the *Allium test* was made by Grant and he has reported 76% of the chemicals with a definite positive response. Allium test is included among the routine tests used for assessing chromosomal damage induced by chemicals.

A. Effect of Pesticides on *Allium cepa*

Use of pesticides has become unavoidable for crop protection in modern agriculture. The frequent and indiscriminate use of these chemicals results in many undesirable secondary consequences on higher plants (Epstein and Legator, 1971; Amer and Farah, 1974). Genotoxic effects of some organophosphorus peticides on *A. cepa* root meristems

were assessed by Grover and Malhi (1988). The mutagenic effects of insecticides such as Malathion, Fensulphothion, Phosalone, Lindane, Parathion, Methyl Parathion, Trimiltox, Aldrex-30 etc. on *A. cepa* cells have been investigated by different workers (Mishra and Sinha, 1979; Panda and Sahu, 1985; Sinha *et al.*, 1989; Kumar and Sinha, 1989; Pratibha devi *et al.*, 1991; Saeed and Robina, 1992; Pandey *et al.* 1994). A number of herbicides such as Nitraline, Isoproturon, Stomp, Glean, Dual, Asulum, MSMA, Igran, Tribunil, Garlon-4, Sencor, Saturn etc. were also found to induce antimitotic activities in the cells of *A. cepa* (Badr, 1979, 1983; Badr and Elkington, 1982; Mousa, 1982a; Badr and Ibrahim, 1987; El-Boyoumi *et al.* 1987; Rao *et al.*, 1988; El-Khodary *et al.*, 1987, 1989, 1990; Haliem, 1990; Chauhan and Sundararaman, 1990; Ignacimuthu and Saravankumar, 1991). Cytological aberrations during fungicidal treatments are reported by Mohan (1975), Somasekhar *et al.* (1984), Badr (1988), Pandey *et al.* (1994).

B. Effect of Growth Regulators

Certain chemicals stimulate or induce mitosis in plant cells. The plant growth regulators such as IAA are known to stimulate mitosis in *A. cepa,* root tip cells at lower concentration (Singh, 1982). However sublethal concentrations of IAA, NAA, GA, Kinetin etc produce one or

more cytological aberrations in *A. cepa* roots (George, 1972-73). Abscisic acid is also reported to induce a number of mitotic abnormalities in *A. cepa* (El-Nahas, 1989). Effect of Maleic hydrazide on root mitosis has been investigated by Mann *et al.* (1974), Oku (1977), Singh (1982), Edwin *et al.* (1993) etc. These effects are sdiscussed further in the present study.

C. Effect of Other Chemicals

Chemical mutagen MNNG can induce chromosomal aberrations in *A. cepa* (Grover and Dhingra, 1987). Several other chemicals such as caffeine, Pyronin Y, ∝-amanitin, Actinomycin-D have been tested on meristematic cells of *A. cepa* and are reported to induce various degrees of cytological abnormalities (Batikyan *et al.* 1973 a and b; Torre *et al.*, 1974; Cortes and Hazen, 1984). Clastogenic activity induced by heterocyclic compounds Quinoline, Isoquinoline and Pyridine has been examined by Abraham *et al.* (1990). Cytogenetic effects of organic mercury compounds (Ramel, 1969), calcium salts (Mishra, 1982), Single super phosphate and urea (Chaurasia and Sinha, 1986, 1987) on mitotic chromosomes of onion have been investigated. Effects of detergent, sodium lauryl sulphate on pollen mother cells of *Allium cepa* are studied by Datta *et al.* (1988). El-Nahas *et al.* (1988) have reported meiotic disorders induced by Nadolol in *A cepa* pollen mother cells.

D. Effects of Plant Extracts

Recently medicinal plants are used increasingly for curing a number of disorders in human beings. Existance of carcinogenic properties in the medicinal plants can never be ruled out. Evaluation of crude extracts of plants for cytological effects is very essential to avoid harmful effects of these plants. Keck and Hoffmann-Ostenhof (1952); Kato (1957); Tarkovska (1971); Shehab (1980) Shehab and Adam (1983); Shehab *et al.*, 1978) Patnaik *et al.* (1984) have made significant contribution in this field. An alliaceous juice' Alliostabil' caused shrinkage and vacuolization of cytoplasm, disappearance of mictochondria and proplastids and various mitotic disturbances in *A. cepa* roots (Grzycka and Obuchowska, 1971). Water extract of *Ipomoea carnea* has also proved to be mitodepressive (Alam *et al.*, 1987). Cellular damage in *Allium cepa* root meristem induced by leaf extract of *Ricinus communis* was investigated by George and Geethamma (1990). Many physiological and clastogenic aberrations induced by leaf homogenates of *Tylophora indica* on *Allium cepa* were observed by Saggoo *et al.* (1991). Antimitotic effects of water soluble saponine holothurin on *Allium cepa*, causing chromosomal clumping, nuclear pycnosis, chromatin extrusion are also known (Santhakumari and Stephen, 1988). Different types of tobacco extracts can be mutagenic in action at certain

concentrations (Banerjee, 1992). Certain food dyes and essential oils were also found mitodepressive and produced usual chromosomal aberrations in *A. cepa* (Roychoudhury and Giri, 1989; Puerta *et al.* 1993). Similar mitotic effects of shoot decoction of *Ephedra foliata* have been recorded by Saggoo *et al.* (1993) in *A. cepa* root tips. Chromosomal damage produced by the ediable fruit pulp of *Tamarindus indica* in onion cells was observed by Susan and Pillai (1979). Mutagenic efficiency of Cyclophosphamide, maleic hydrazide etc. was activated by plant microsomal extracts prepared from young maize seedlings (Darroudi and Natarajan, 1987).

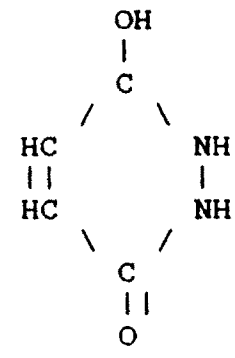
E. Miscellaneous Effects

Runthala and Bhattacharya (1991) observed a significant fall in mitotic index and induction of various mitotic and meiotic abnormalities in *A. cepa* cells exposed to static and electromagnetic field treatment. X-ray induced chromosomal aberrations in root tip cells of *A. cepa* have been reported by Sax (1941). Mitotic abnormalities induced by tobacco smoke condensate in onion root tips have been reported by Bhalla *et al.* (1973).

3. **MALEIC HYDRAZIDE : A Growth Regulator**

Chemical Name - 1,2-dihydropyridazine-3,6-dione

Chemical Structure



Type - Growth inhibitor and a selective translocated herbicide

Trade Name - Maleic hydrazide, Maleic hydrazine, MH,

Properties - It is a white crystalline solid having acidic properties with 6000 ppm solubility at 25 ^{o}C. The diethanolamine salt is water soluble

M.W. - 112.1

LD50 - 4000 mg/kg

Formulation - Emulsifiable liquid and wettable powder.

General Dosage - 3 to 6 kg/hac.

A. ***Applications***

Maleic hydrazide is widely used as a growth rgulator, growth inhibitor and weed killer. It is used to control the blossoming of certain horticultural crops, for reducing growth of hedges, lawns and to prevent sucker

production in tobacco, cotton. It causes retardation of growth of grasses. It also arrests sprouting in stored root crops like potatoes, onion, carrot, beet and prevents flowering in sugarcane. As a herbicide it is effective in controlling perennial grasses such as quackgrass, burmudagrass, johnsongrass. Foliar spray solution containing 1000 ppm MH has been used successfully to kill *Heleotropium indicum* a common weed of India (Panigrahi and Misra. 1957. MH can be applied both for pre and post emergance treatments.

B. *Mode of Action*

MH is absorbed through foliage. It apparently inhibits growth by inhibiting cell division. It disturbs and reduces mitotic activity, as reported by Darlington and McLeish (1951) and Carlson (1954). A change in plasma viscosity leading to spindle arrest and chromosome clarification has been recorded by Sharma and De (1956). MH possibly injures the sieve tubes and therefore, interferes with the translocation. Girolami (1951) observed phloem necrosis caused by MH. The chemical may block hydrolysis or photosynthesis and modify the respiratory activity of the plant. The death of plants is possibly due to starvation as a result of prolonged dormancy or by phloem malfunction which affects the translocation process.

C. *General Growth Response*

Maleic hydrazide has long been recognized as an inhibitor of plant growth. Naylor and Davis (1950) observed uniform growth effects in many mono and dicotyledonous plants treated with MH. Biological properties of MH were discovered by Schoene and Hoffman (1949). It is readily translocated in plants (Crafts, 1959) and inhibits cell division but not cell extension. Its action appeared on cell division in both the apical and subapical meristems of the shoot (Sachs and Lang, 1961). Margaret and Seltmann (1983) studied anatomy of axillary meristems from tobacco plants treated with MH and found that MH controls growth by inhibiting cell division. Gifford (1956) observed aberrant nuclei in the intercalary plates. MH treatment is used to retard vegetative growth and enhance flowering (Kojima and Maeda, 1958). Cytological studies carried out by Scott (1968) provide evidence for inhiition of DNA replication by maleic hydrazide. Physiological response given by plants to maleic hydrazide has been discussed further along with the findings of present investigation.

D. *Residual Effect*

It is leached or decomposed readily in the soil hence residue life is very short. For onion and potatoes 15 to 20 ppm MH concentration is established for application.

4. SCOPE OF PRESENT INVESTIGATION

Maleic hydrazide is widely used as a growth inhibitor and a weed killer. Being a selective translocated herbicide, it is absorbed quickly and moves readily through the plant. The inhibition of growth occurs due to its effect on cell division. In the present investigation action of Maleic hydrazide on cytology and physiology of two onion varieties has been investigated. Onion is considered as an excellent plant for the study of cytological abnormalities induced by chemical treatment. In order to analyse the response given by different varieties of onion, two common varieties were selected in the present study. Usually root cells of bulbs are used for cytological studies but in the present investigation, seeds treated with Maleic hydrazide have been used for this purpose. The study of physiological response of *Allium cepa* to the MH treatment carried out in this investigation may add to the present knowledge of the herbicidal effect on plants. Meiotic studies in the plant raised from MH treated seeds may be helpful in knowing the extent of action of MH.