I REVIEW OF LITERATURE

The use of pesticides for control of pests and diseases of crops is one of the critical inputs for stepping up the agricultural production. Annually 4,644 M. tons of technical grade pesticides are being used in the state of Maharashtra (Bulletin Govt. of Maharashtra, 1985). According to the Bulletin on plant pathology extension (Plant Pathology Courier Vol. 5(1), 1987) the demand of pesticides for 1987-88 reported to be 75,545 M. tons. In Maharashtra during the year 1983-84 large scale of plant protection campaign against important pests of crops were organised over an area of 8.5 lakh hectares. When all out efforts are being made to maximise agricultural production such as use of hybrid and high yielding varieties of crops and adoption of improved agricultural technology, it is needless to emphasize the necessity of undertaking large scale plant protection measures to save huge annual losses caused due to vagaries of pests and diseases.

Though the consumption of pesticides for protecting the crops from the attack of pests and diseases has increased considerably, there is necessity for guiding the cultivators to use right type of pesticides for control of different pests and diseases. Large number of pesticides put forth by various commercial firms are available in the market, several new products are being added annually. It has often experienced that cultivators find it difficult to select proper pesticides for pest and disease control or otherwise without knowing the danger of pesticides they use heavy doses which ultimately results into health hazards.

In order to provide information regarding the suitable pesticides against crop pests and diseases, the time of application, precautionary measures to be taken while handling the pesticides, etc. has been incorporated into number of books and booklets. Since the problems of plant-protection have become particularly important in recent years, the scientists are engaged in studying the after effects of pesdticides in seed germination, metabolic activities in plants, residual problems and cytotoxicity.

A. Effect of Pesticides on Germination and Growth

Pesticides have become an essential part of plant protection. Among the vast number of plant protection chemicals some exhibit favourable effects such as better fruit set, seed set, etc. (Philomena and David, 1985). However, some have shown to cause toxic effects on plants in various ways (Stoker,1948; Puchkova, 1965; Casida and Lykken, 1969; Buczacki and Cadd, 1976; Prasad et al. 1980). In all these studies however, their effect on seed germination seems to have been overlooked. But in order to get complete understanding of the plant pesticide relationship several workers have undertaken such type of study.

Prasad and Mathur (1983) have studied the effect of Metasystox and Cuman-L on seed germination of Vigna mungo. Their study revealed that Metasystox inhibited seed germination at all the doses viz. 0.5, 0.8, 0.10, 0.15% while Cuman-L on the other hand had no or little effect on seed germination. According to Singh et al. (1979) in general insecticide treatments did not reduce germination (except Ambithion) but did reduce seedling height, pollen fertility and chiasma frequency in Hordeum vulgare. Effect of various concentrations of Sevin on growth parameters of mungbean (Vigna radiata) seedlings have been reported by Pathak and Mukherji (1986). They found that root and shoot length increases with increase in concentration but higher concentration inhibits the growth of root and shoot. According to them Sevin can be used at a concentration of 100-200 ppm with no detrimental effects. Treatments with pesticides are known to reduce germination and seedling height (George et al. 1970; Epstein and Legator, 1971; Tomkins and Grant, 1972; Mote, 1978; Chakraborthy et al., 1983) are more common. Recently Sengupta et al. (1988) have also studied the carbaryl toxicity in germinating seeds of Vigna sinensis, and have reported that gibberellic acid on simultaneous application with toxic doses of carbaryl, overcome the growth inhibition caused by low concentration of carbaryl. A gradual decrease in the germination percentage of Dolichos biflorus with increased concentrations of Kitazin (a fungicide) has also been reported by Reddy and Vidyavati (1983).

Krishnamurty and Rao (1980) have also reported that the increasing concentrations of Antracol and Kitazin gradually inhibit germination, seedling growth and plant height in Brassica nigra. Seeds treated with a potential insecticide Sumithion showed leaf variation from the normal trifoliate condition in control to tetra, penta, heptafoliate condition (Krishnamurty and Rao, 1980a) in treated plants.

Effect of Isoproturon (herbicide) on germination and early seedling growth of canary grass has been studied by Jaiprakash and Singh (1984) and reported that varying concentrations of Isoproturon inhibit the germination percentage and root, shoot length. Agrawal and Soam (1988) have also studied the effect of Thiodan 35 E.C. on germination and growth of pea. They have reported that 1% Thiodan adversely affects radicle and plumule length. The more visible symptoms of toxicity found by them were coiling and blackening of radicle tips.

Effect of different pesticides on germination and seedling growth in wheat (Sachan et al. 1967), Chick pea (Shrista and Kale, 1970), Cotton (Gawaad et al. 1972), Sugar beet (Sinha et al. 1981), *Sesamum indicum* (Kamala and Rao 1982), *Vigna mungo* (Chandra et al. 1983), *Vigna radiata* (Gupta et al. 1983), hulisoppu, sunnhemp, ragi, sunflower, jowar, greengram, bajra, pea, maize, (Somashekhar and Sreenath, 1986), coffee seeds (Venkatasubbaiah and Safeeulla 1987) has been reported. Further, inhibitory effects on early germination phase in sorghum, finger millets, rice and cluster beans due to seed soaking with 0.5% Mevinphos, Demeton, Schradan and Thiometon have also been reported by David (1965).

B. Effect of Pesticides on Enzyme Activity

Since pesticides are being used during the recent years extensively in the developing countries, the adverse effects as produced by these chemicals are also the subject of growing concern today (Tatekar et al. 1977). Although there are several reports on the effects of pesticides on the seedling growth, biochemical studies regarding pesticide mediated changes in germinating seeds are relatively scanty (Guha et al. 1987). Therefore, it is alarmingly important to screen the seeds/plants for biochemical effects of almost all the chemicals being used commercially. Some of the biochemical work carried elsewhere as regards the pesticidal effect on enzyme activity is reviewed below.

Arnison and Boll (1976) have studied the effect of 2,4-D and Kinetin on peroxidase activity and isoenzyme pattern in cotyledon cell suspension cultures of bush bean (Phaseolus vulgaris) and shown that the activity of peroxidase was minimum during the phase of cell division and maximum during the phase of cell expansion. Similarly, Reddy and Vidyavati (1983) have studied the effect of Kitazin a fungicide on the hydrolytic enzymes in Dolichos biflorus seedlings. They have reported that the activity of amylase, protease and catalase decreased at higher concentration but slightly stimulated at lower concentration of Kitazin. Contrary to this the peroxidase activity was reported to be less at lower concentration and more at higher concentration.

Prasad and Mathur (1983) have studied the effect of Metasystox and Cuman-L on the activity of enzyme amylase in the germinating seeds of Vigna mungo and reported that the inhibition of amylase activity even with little or no effect due to Cuman-L treatments. The toxic effects of carbaryl on the activities of different hydrolyases of germinating Vigna sinensis seeds have also been investigated by Sengupta et al. (1988). According to them the activities of amylase and phytase from cotyledons decreased on exposure to carbaryl but ATPase extracted from seedlings showed stimulation followed by carbaryl application. Further, Pathak and Mukharji (1986) have also studied the effect of carbaryl on the activity of enzymes amylase, protease, RNase in germinating seeds of Vigna radiata. Their study revealed that carbaryl can be used at a concentration of 100-200 ppm with no detrimental effects. The influence of Dimethoate on deoxyribonuclease and ribonuclease in the Vigna sinensis seeds during germination has been reported by Guha et al. (1987). According to them Dimethoate presumably increases the activation and/or de novo synthesis of DNase in the cotyledon but inhibits the process in the seedlings. On the other hand it stimulates the RNase activity both in cotyledons and seedlings. Reports are also available on the stimulation of the activities of plasma membrane bound ATPase and some hydrolytic enzymes e.g. DNase, RNase in Vigna sinensis by organophosphorus pesticides (Chakraborti et al. 1979, 1981).

Apart from the study of pesticide effect on seedling growth and hydrolytic enzymes most of the workers have studied the effect of foliar application of fungicide on physiology of plant (Singh and Kang, 1978; Kaur and Grover, 1983; Kumar and Khan, 1983).

Recently Karadge and Karne (1985) have studied the influence of systemic fungicides, Bavistin and Calixin on Lycopersicon esculentum leaves. Their data revealed that both the fungicides caused an increase in free organic acids, polyphenols and chlorophylls in the leaves. The total nitrogen and proline contents were decreased and the activity of the enzymes catalase, IAA oxidase were elevated while peroxidase and acid phosphatase were decreased down.

Manoharan et al. (1981) have investigated the effect of Fluchloralin a pre-emergent herbicide on some of the metabolic processes of Vigna unguiculata seedlings. According to them, the herbicide affects the activity of hydrolytic enzymes and alters the rate of respiration and permeability of cell membrane. Apart from these studies the effect of herbicides (Namdeo and Dube, 1973; Bollag and Henninger, 1976; Tui, 1981; Malkomes and Wohler, 1984) and fungicides (Karanth et al. 1975) on soil enzymes are also known. Most of the workers are also engaged in studying the effect of pesticides on soil microflora (Agnihotri, 1973; Tui, 1981; Malkomes and Wohler, 1984), bacterial population (Pandya, 1983) and nitrogen fixing blue green algae (Singh and Vaishampayan, 1978; Singh et al. 1979; Maharana et al. 1986). In addition to this the research on effect of certain pesticides on nodulation of cowpea (Research note; Pesticides, XVIII (8), 1984) and *Pisum sativum* (Reddy *et al.* 1986) is in progress.

The effect of pesticides on the growth and metabolism of *Azatobacter chrococcum* has also been carried out by Balasubramanian and Narayanan (1980). Similar studies by using different pesticides have been reported by many workers. Kulkarni *et al.* (1974) have studied the symbiosis of *Rhizobium* sp. with *Arachis hypogea* under the influence of soil applied insecticides. Effect of pesticide on rhizosphere microflora of cowpea has been investigated by Palaniappan and Balasubramanian (1986).

The effect of Monocrotophos and Dimethoate on certain anatomical details of onion leaf tips (Srinivasan *et al.* 1985) and effect of Sumithion on leaf variations in *Phaseolus vulgaris* (Krishnamurty and Rao, 1980a) have also been carried out. In the former the leaf tip anatomy of onion indicated more protein bodies per individual cells of palisade layer which they accounted as a beneficial effect. While in the latter altered plant metabolism resulted changes in the leaf morphogenesis.

Certain unusual features like multiple pollen tubes, branching of pollen tube due to pesticide application have also been reported by Philomena and David (1985). Salgare (1983) has also reported pollen tube deformities and pollenicidic action brought in by herbicides like 2 4 D, 2.4.5 T and Triazine.

With the development and successful application of antibiotics for control of disease in men and animals there has been continuous search for antibiotics for the control of plant diseases too (Chattopadhyay, 1980). With this view in mind Bose et al. (1982) have studied the effect of chloramphenicol and cyclohexamide on the germination and protease activity in maize seeds. Their study reveals that both the antibiotics inhibit germination and protease activity in endosperm, the latter being more effective than former.

C. Effect of Pesticides on Chromosomal Behaviour

Pesticides are designed to be toxic in order to ensure public health and to protect the raising agricultural outputs. However, they are injurious to human health through i) treatment of consumables in the field like fruits, leaves, and during grain storage, ii) inhalation by the workers in industry and agriculture and, iii) indirect exposure via food chain. These injuries may be genic, chromosomal and/or genomic leading to mutagenicity, clastogenicity and turbagenicity. Yet the pesticides are absolutely indispensible. If not used will probably have famines and foods highly contaminated by fungal toxins. If we use intensively, they will create a problem of health hazards. Therefore, the need to be used judiciously. This is not a new point and has been stressed often. What is probably new is a suspected spectra of mutation, pollution in the agroecosystem that could render the balance in nature chaotic in addition to the consequences hinted earlier. Recent studies on cyto and chromatoxic effects of agro and environmental chemicals and their possible genotoxic effects have prompted several international agencies to recommend the screening of mutagenic and chromotoxic effects of almost all the chemicals being used commercially (Alacevic, 1980; Kaur and Grover, 1985). Therefore, a survey of the salient features of pesticide genetic toxicity relevant to the agricultural system and their consequences may be desirable. The studies carried out so far reviewed below.

About 400 basic pesticidal chemicals are in use all over the world constituting about a few thousand formulations on a conservative reckoning (Sharma, 1986). Other estimations range between 800 to 1500 basic chemicals and 100,000 formulations (Kurinnyi, and Piliniskaya, 1974; Ridgeway et al. 1978) It is also estimated that about 20 billion tons of them are used annually around 1975 (Culliton, 1978) in the USA alone which is the most premier consumer in the world. In India about 80 pesticidal chemicals are in use. The impact of the pesticides on the genetic material of various organisms has been known for some time accumulating a great mass of literature (Sharma, 1986), 20% of which deal only with plants. While the estimates may vary from worker to worker and subject to periodical revision (Kurinnyi and Piliniskaya 1974).

As early as in 1937 Sass made an anatomical study of hypertrophy induced in maize by new improved Ceresan (Ethyl mercury phosphate). He showed that the primordia undergo extensive thickening and develop irregular carnations and lobes and in the apical meristem of the plumule, cell division is inhibited and extreme enlargement of the existing cells takes place. The cells of the hypertrophied organs become multinucleate, containing nuclei ranging from 'micro nuclei' to polyploid 'giant nuclei' and the number of chromosomes in these often exceeds 200. Sass (1937) considered the malformation to be due to incomplete meiosis.

Vaarama (1947) has conducted an experiment to study the influence of DDT insecticide upon plant mitosis. Crocker (1953) has studied the effects of 2-4 dichlorophenoxy acetic acid and 2,4-5 trichloro phenoxy acetic acid on mitosis in Allium cepa. Scholer (1955) carried out an experiment to study the effect of Dieldrin, Aldrin, Isodrin, Endrin and DDT on mitosis in onion root tip. Amer (1965) reported arrested mitosis due to insecticide treatment. The chromosomal aberrations induced in somatic cells of Vicia faba by pesticides and the occurrence of morphological and somatic chromosome aberrations caused due to pesticides in barley are well known (Wuu and Grant, 1966, 1966a). Amer and Ali (1968, 1969) have studied the cytological effects of pesticides on meiotic as well as mitotic cell division. Cytological effects of pesticides on Vicia faba have also been reported by Amer and Odette (1976).

Sax and Sax (1968) have observed the chromosomal breakages in onion root tip cells due to Dichlorovos treatment. The mitotic depression and a high frequency of chromosomal aberrations in barley are also caused due to the action of Dichlorovos (Bhan and Kaul, 1975). Similarly Ayling (1977) has studied the effect of Tordon herbicide on Pinus seedlings and reported aberrations in the cell division. Sathaiah et al. (1974) have studied the cytological effects of Carbofuran and observed many chromosomal irregularities.

Kurinnyi (1975) has done a comparative investigation of cytogenetic effects of certain organophosphorus pesticides. Similarly Zutshi and Kaul (1975) studied the cytogenetic activity of common fungicides in higher plants. The comparative cytogenetic effect of Aldrin and phosphamidon has been reported by Georgian (1975). Wild (1975) tested the organophosphorus insecticides for mutagenicity.

Mann (1977) studied the cytological and genetical effects of Dithane fungicides on Allium cepa and reported the abnormal behaviour of chromosomes in treated plants and concluded that reproductive cells are more sensitive than somatic cells as higher frequencies of chromosomal aberrations observed in meiotic cells. The author pointed out that the erratic behaviour of chromosomes at meiosis induced by the fungicides caused pollen sterility and lowering of seed set.

Singh et al. (1979) have also conducted an experiment to study the effect of insecticides on behaviour of chromosomes in barley. They observed different cytological aberrations such as chromosome fragments at mototic metaphase; chromatin bridges, fragments and/or laggards at mitotic anaphase; univalents, precocious separation and non orientation of bivalents at meiotic metaphase I and such several abnormalities in treated populations, some insecticides induced significantly greater cytological aberrations and other detrimental effects. Cytogenetic aberrations induced by nine different insecticide treatments in barley seeds have been reported by Singh et al. (1977). They observed many abnormalities like chromosome fragments, non-orientation of chromosomes at metaphase, laggards at anaphase in root tips.

Mishra et al. (1979) have studied the effect of Malathion on mitotically dividing onion (Allium cepa) root tip cells and reported abnormalities like laggards, stickiness, anaphase and telophase bridges, multipolar spindles. Similarly Sawamura (1965) examined 12 non-harmonic herbicides on the hair cells of Tradescantia, the stipular hair of Vicia and pollen grain and mitosis of Tradescantia. Suppression of spindle development, coagulation of protoplasm, mitotic aberrations and inhibition of mitosis were reported due to different herbicide actions.

Grover and Tyagi (1980, 1980a) have reported spectrum of abnormalities caused by some common pesticides like Thiodon, Folithion Lebaayacid and

Kitazin in barley. Cytogenetic hazards from agricultural chemicals have also been reported by Panda and Sharma (1980) in Hordeum vulgare. Bhamburkar and Bhalla (1980) have studied the differential mutagenic sensitivity of three varieties of Allium cepa.

Shoemaker and Aihrke (1983) have investigated the effect of pesticides on recombination in maize and reported the ability of pesticides to increase the frequency of genetic recombination between markers of maize, which supports the consensus that certain pesticides at abnormally high rates of application are genetically active environmental recombinogens. Cytogenetic hazards from agricultural chemicals have been studied by Sahu et al (1983) and reported the clastogenic effects of some fungicides on root meristems of Allium cepa, Allium sativum and Vicia faba. Fujii and Inoue (1983) have examined several agricultural chemicals for their mutagenicity in the soyabean test system.

deKergommeaux et al. (1983) studied clastogenic and physiological response of chromosomes to different pesticides in Vicia faba in vivo root tip assay system. Effect of agrochemicals on mitotic chromosome behaviour in tetraploid and hexaploid wheats has also been studied by Halvankar and Patil (1984). Similarly Rahman and Bhattacharya (1985) have conducted an experiment and studied the comparative cytological effects of Sevin on vegetatively and sexually reproducing plants Allium cepa and Lathyrus sativus and concluded that the two plant taxa are vegetatively and other sexually reproducing possess differential response to insecticide Sevin. Differential effects of pesticides on a vegetatively reproducing plant Caladium bicolor has been studied by Rahman et al. (1986). They observed abnormal mitosis as well as chromosomal irregularities, increased regularly with the increase in concentration of pesticides and in some other cases, increase in the period of treatment increased the percentage of abnormalities. In Sevin treated root tips polyploidy and scattered metaphase were observed whereas Atrazine produced subnarcotic effects (bridges, fragments, clumping). They concluded from these experiments that the same plant responds differentially to the two chemicals studied.

Rao et al. (1986) have studied clastogenic and turbagenic effects of organophosphorus pesticides namely Malathion, Methylparathion and Quinolphos on Allium cepa root meristems, and reported that methylparathion is most effective clastogenic as well as turbagenic agent. Where as Quinolphos is weak clastogenic and turbagenic agent. Padmaja et al. (1986) worked on the effect of Dimecron on somatic cells of Allium sativum and reported the fall of mitotic indices due to Dimecron treatment. They have also observed several mitotic abnormalities like scattering of chromosomes, formation of diplochromosomes, sticky metaphase, disturbed anaphase giving rise to tri, tetrapolar cells, nuclear pycnosis and chromosome clumping and clastogenic effects such as fragmentation breaks bridges, bi, tri and tetranucleate cells indicating the inhibition of cytokinesis. Similarly Somashekhar (1986) has also noted the inhibition of mitotic division to some extent by the action of fungicide Metaxyl on the root meristem of Zea mays. He also observed several anomalies like thinning of nuclear material, nuclear vacuolation, precocious movement, diagonal spindle and poles, elongation of nuclei, C metaphase, stickyness, bi and multinucleate cells, breaks and gaps etc. Recently Halvankar and Patil (1987) have made an extensive study on the effect of some agrochemicals like Dithane Z-78 and Deconil 2787 (fungicides) Sevin, BHC (insecticides) and 2,4-D (weedicide) on meiotic chromosome behaviour in tetraploid and hexaploid wheats, and reported that hexaploid wheats appeared to be more affected than tetraploid wheats.

Above all, the study in relation to persistance of pesticides in soil, water and sediment is equally important and has been reported by Agnihotri and Jain (1987) and Agnihotri et al. (1987).

D. Summary of Workdone and Scope of Present Investigation

The problems of plant protection have become particularly important in recent years because of the threat to human health and the environment through the large scale use of pesticides (Finlayson and MacCarthy, 1973). Methylparathion an organophosphorus pesticide is widely used as an insecticide in the form of spray whose residual effect is known to remain in the environment for long time (Deshpande and Swamy, 1987; Sharma and Chopra, 1970). Moreover most of the pesticides have been shown to cause toxic effects on plants in various ways (Casida and Lykken, 1969; Prasad and Mathur, 1983; Karadge and Karne, 1985; Deshpande and Swamy, 1987).

Pesticides also play a very important role in protecting vegetables, fruits and food grains right from the seed stage through germination to growth and then storing (Mann, 1977). However, their use has many consequences (Bernard, 1963; Brown, 1962, 1963; Cottam, 1965) and from studies of many environmental chemicals, biocides, the conclusion has been reached that they can induce considerable damage into the human gene pool (Grant, 1970 and 1971). Recent studies on cyto and chromotoxic effects of agro and environmental chemicals and their possible genotoxic effects have prompted several international agencies to recommend the screening of mutagenic and chromotoxic effects of almost all the chemicals being used commercially (Alacevic, 1980; Kaur and Grover, 1985).

Most plants respond to changing osmotic potentials in their external environment by osmotic adjustments of their cellular content (Maas and Nieman 1978; Greenway and Munns, 1980). Both inorganic ions and organic compounds are utilised for this purpose. Proline is one of the organic compounds that has been reported to accumulate in plants subjected to water stress (Aspinall and Paleg, 1981; Patil and Hegde, 1983), Salinity (Goas et al. 1982; Weimberg et al 1982; Naik and Joshi, 1986) nutrient deficiencies (Ghildiyal et al. 1986) Water logging (Wample and Bewley, 1975), fungal infection (Sinha et al.,1984) and air pollutants (Soldatini et al.,1978). Though an osmoregulatory role for proline at the cellular level has been suggested (Stewart and Lee, 1974), the significance of its accumulation has not been unequivocally established (Goas et al., 1982). Since number of stress factors known to induce proline accumulation very little (Deshpande and Swamy, 1987) information is available on the induction of proline accumulation by pesticidal stress.

From the above foregoing literature survey it is very clear that, in most of the experiments, the routine plants like onion, barley and mung bean have been used for studying cytotoxicity caused due to pesticides. Similarly the seeds of wheat, jowar, maize and mung bean have been used for studying pesticidal effect on seed germination, seedling growth and to some extent on the enzymes involved in germination. However, not much information is available about the vegetable seeds of day-today diet such as kidney bean, okra, guar and onion as regards to the effects of Metacid-50 and Dimecron on seed germination, seedling growth, cytotoxicity, hydrolytic enzymes and proline content. Keeping this view in mind, the effect of widely used organophosphorus pesticides such as Metacid-50 and Dimecron on the above parameters in seeds of bean, okra, guar and onion have been attempted in the present investigation.