CHAPTER 1

INTRODUCTION

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Modern agriculture has evolved immensely from the simple crop culture to become an environmental technology with its focus on the management of land, water, air and biological resources for the production of food and fibre and for the preservation of natural resources. The farm is, in fact, a managed ecosystem. For successful operation, it must be a well regulated ecosystem where the renewable resources are effectively conserved and harmful outputs are carefully minimized. Pimentel and associates (1970) mention: "More than ever before in man's history it is imperative that intensive research be utilized to develop the technology by which agricultural practices can more effectively conserve our vast land, water and biological resources."

1-1 Ecological backlashes of modern agriculture:

In spite of all this understanding, modern agriculture, particularly the highly industrialized agriculture as practised in Japan and other countries, is becoming an unmanaged monstrosity. Odum (1971) discussed the ecological implications of modern agriculture. Two of his main conclusions deserve special mention — (1) "....the agricultural and the forestry specialist must now consider that his crops have outputs other
than food and fiber in terms of man's total ecosystem and (ii) "maximizing for yield without regard to other consequences is producing very serious backlashes, both environmental and social." In order to double the crop yield in cropland ecosystem requires a ten-fold increase in fertilizers, pesticides, and horse power. The industrialized (fuel powered) agriculture produces four times the yield per acre as does man-and-domestic-animal powered agriculture (as in India), but is hundred times as demanding on resources and energy. Thus, agro-industry is one of the chief causes air and water pollution.

1-1-a Hypothetical input-output model of a cropland:

Fig. 10 is a hypothetical model of various inputs in an agricultural system and outputs which emanate from such a system. "It is clear that in terms of direct outputs the system gives out food and fibre together with non-degradable pollutants like DDT, mercurial salts, etc., carcinogenic chemicals in the form of pesticides, herbicides, fungicides and antibiotics, disease causing organisms, allergy causing agents and, air and water polluting chemicals. In its indirect demand, the modern agriculture puts tremendous pressures upon the non-renewable sources of energy like fossil fuels like petroleum and natural gas, and natural resources
like minerals. In a developing country like India, the growth of modern agriculture puts import pressures as well.

The whole situation is very much aggravated on account of ever increasing pressures of human population explosion which demand immediate increase in the food production. The situation is truly desperate and in the words of Stern, Dobzhansky et al. (1970), "there may be mass starvation on a tragic scale within this century unless there is a prompt and major rise in production; and, therefore, there is urgent need for the application of already existing knowledge."

Basic theory does not result in increased production without a great deal of scientific work on local soil and water, crop management, development of seed strains, animal husbandry, pest control, and more importantly, fertilizer usage.

Most of these input chemicals of modern agriculture have come under severe criticism from different quarters.

1-1-b Pesticides, herbicides and fungicides:

Pesticides, herbicides, fungicides, in spite of their great use in crop management, are the most notorious agricultural backlashes. The increase in the use of these substances is leading to almost senseless saturation of the environment, with the persistent broad-spectrum poisons, to
the point that we now must phase out many of them. This has resulted in many types of ecological backlashes. Solomon (1953) and Ripper (1956) reported the entomological backlash in the form of pest outbreaks induced by agricultural spraying. Carson (1962), in his famous book *Silent Spring*, has dramatically brought to public attention the poisoning of entire food chains by insecticides. Nicholson, Grezenda et al. (1964) demonstrated how whole watersheds become contaminated by the uncontrolled use of agricultural pesticides. The chlorinated hydrocarbons, which are now among the world's most widely distributed synthetic chemicals, are not only contaminating the biosphere but also reaching saturation level in many human and animal populations (Wurster, 1969).

In their harmful effects herbicides are not lagging behind. Herbicides fall into two groups, depending upon their mode of action. Those in the first category, which includes monuron and simazine, interfere with photosynthesis and thus cause the plant to die for lack of energy. The second group is typified by the commonly used 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) which result in defoliation and systematic lethal action. Picloram has been characterized by Galston (1970) as a herbicidal analog to DDT because of its relative persistence in soils. Cacodylic acid contains over 50 per cent arsenic acid and repeated use may lead to build up of arsenic in soils. In the words of Odum (1971),
"Insecticides and herbicides together are powerful drugs in the ecosystem since they modify the function of vital systems - the consumers and producers. It is now being suggested that these substances be under licensed control of trained professionals, just as are drugs used to treat the human body."

1-2 Cytogenetical backlash:

There is another dimension of this problem and that relates to the cytogenetical effects of many input chemical compounds. Many of these chemical substances have been subjected to experimental studies to investigate their mitotic, chromosomal, mutagenic and carcinogenic effects. The following review endeavours a brief survey of some of the more important contributions made in this area during the last decade. This review deliberately avoids a reference to many studies made on the effects of various chemicals on cells, cell division and chromosomes, and concentrates mainly on agricultural input chemicals. For a broader understanding of such studies, some of the notable reviews are those of Sharma and Mokerjea (1954) on cell division stimulatory substances, Sharma and Sharma (1960) on chemically induced chromosome breaks, Mehra (1960) on the effect of chemicals on cell division, Selfant (1963) on cell division
inhibitors, Kihlman (1966) on the action of chemicals on dividing cells, Daysson (1968) on antimitotic substances, Mishra (1975) on the effects of psychotropic drugs on cell division, cell size, and chromosomes.

1-3 A general review of the studies on the cytogenetic effects of the agricultural input chemicals:

The various studies on the cytogenetic effects of different agricultural input chemicals can be reviewed conveniently under following heads —


1-3-a Herbicides:

Doxey (1949) investigated the effects of isopropyl-phenyl carbamate on mitosis in rye and onion. This herbicide produces aberrations in dividing plant cells similar to those induced by colchicine, carbendazim and griseofulvin. Mann and Storey (1966) studied the action of carbamate herbicide upon plant cell nuclei. Two compounds were investigated — IPC and CIPC. According to these workers, these herbicides are
useful in making determinations of plant chromosome number in root tips. Bayer et al. (1967) reported the morphological and histological effects of the herbicide trifluralin. This herbicide also produces aberrations similar to those induced by colchicine, carbendazim and griseofulvin. Hepler and Jackson (1969) studied the effects of isopropyl-N-phenyl carbamate on dividing endosperm cells. The herbicide disrupts the assembly of microtubules. Herichova (1970, 1971) studied the effects of isopropyl-N-phenyl carbamate on chromosome structure and cytokinesis and also the ultrastructural effects on the meristematic cells of barley. According to Herichova, carbamate alters chromosome structure and prevents cytokinesis in the meristematic cells of Vicia faba. The compound shows accumulation of C-metaphase, spiralized chromosomes, polyploid nuclei, micronuclei and binucleate cells. IPC also caused fragmentation of the endoplasmic reticulum and golgi apparatus. Guenther and Nasta (1972) studied the effects of the carbamate compound CIPC on mitosis of Allium cepa and Hordeum vulgare. This herbicide inhibits mitosis, mitotic spindle and causes stickiness and pycnosis of the chromosomes. Jackson and Stetler (1973) reported the effects of the herbicide trifluralin on the regulation of mitosis. The herbicide prevents the assembly of microtubules. Bartels and Hilton (1973) made a comparison of trifluralin, bryzalin, pronamide, propahm and colchicine treatments on microtubules. This study also reveals the disruption or
prevention of the assembly of microtubules. Sharma (1973) reported meiotic anomalies caused by herbicide simazine, embutox and atrazine. These herbicides induced fragments, laggards and caused stickiness. Hakeem and Shehab (1974a, 1974b) investigated the effects of dalapon, gramoxone and 2,4-D amine on *Vicia faba* cells. These herbicides induced mitotic and meiotic anomalies like stickiness, bridges, laggards, fragments, polyploidy etc. Hess and Bayer (1974) have reported the effects of trifluralin on dividing cells of cotton roots. The herbicide induces aberrations similar to colchicine, carbendazim and griseofulvin. Bakale (1976) reported the effects of weedicides on cytology of *Xanthium strumarium* L. Mohandas and Grant (1972) and Singh and Harvey (1975) studied the cytogenetic effects of weedicide 2,4-D. This herbicide induces chromosomal bridges, fragments, laggards and chromosomal contraction. Seed treatments of *Hordeum vulgare* show that 2,4-D induce albino mutants. Singh and Harvey (1975) on the basis of studies on suspension cultures of *Vicia hasjitana* and *Haplopappus gracilis* found that the induced mitotic anomalies are negatively associated with 2,4-D concentration.

It is clear from these studies that most of the herbicides are toxic to mitotic cycle and induce somatic and mitotic chromosomal anomalies.
1-3-b Pesticides:

Vaarama (1947) studied the effects of DDT insecticide upon plant mitoses. Pure DDT was observed to have a weak C-mitotic effects. This effect got aggravated when DDT treatment was given with ethyl alcohol and resulted in C-mitotic chromosome contraction, multipolar spindles, polyploid cells and lagging nucleoli. D’Amato (1949, 1950) reported induction of chromosome breaks and polyploidy by pure gammexene. Scholes (1953, 1955a and 1955b) reported the effects of hexachlorocyclohexane, chlordane and toxaphene, aldrin, dieldrin, endrin and DDT on mitosis in roots of Allium cepa and Fragaria vesca. These pesticides caused a wide variety of cytological and chromosomal effects ranging from lethal effects on resting cells to metaphase arrest and chromosome breaks. Amer et al. (1965, 1966a, 1968b, 1969, 1971 and 1974) studied the effects of N-methyl-1-naphthyl carbamate, phenol and Rogor. Carbamate induces mitotic and meiotic anomalies like stickiness, chromosome laggards and polyploids. Phenols which are used as pesticides and are also the degradation or end-products of well known pesticides, induce stickiness, laggards, bridges, and fragmentation in meiotic chromosomes. The pesticide rogor inhibits the cell division and induced chromosome stickiness, fragmentation, anaphase bridges and multipolar anaphases. Reddy and Rao (1969) investigated the effects
of insecticides dimecron-100 and roger-40. These systematic insecticides cause mitotic and meiotic chromosomal abnormalities. Mitotic aberrations include chromatid breaks, dot deletions, fragments and bridges. Meiotic aberrations include fragments, ring chromosomes, bridges, laggards and tetraploidy. Wuu and Grant (1966, 1967a and 1967b) reported the somatic and meiotic chromosomal aberrations caused by a variety of pesticides. Fifteen pesticides were investigated and included such well known herbicides alanap-3, atrazine, banvel-3, cytrol, embutox-3, hyvar-X, lorox, monuron, simazine; insecticides like endrin, phosphamidon, sevin, ENT-50612 and botran. All these pesticides were shown to be capable of causing chromosomal aberrations and in certain cases abnormal cellular behaviour, such as cytoplasmic furrowing. The chromosomal aberrations included stickiness, clumping, bridges, fragments and micronuclei. Grant (1972) in an interesting paper entitled as, "Pesticides—subtle promoters of evolution," reported the mutagenic effects of various pesticides. Ramel (1969) reported the genetic effects of organic mercury compounds on Allium roots. Ahmed and Grant (1972) reported the effects of phosdrin and bladex: Tomkins and Grant (1972) observed the effects of pesticides menazone, metrobromuron and tetrachloroisophtalonitrile on Hordeum and Tradescantia chromosomes. All these pesticides caused chromosomal abnormalities like fragments, bridges, multipolar anaphases, laggards and are toxic to cells. In
an important contribution, Kilgore and Ming-Yu Li (1973) reviewed the carcinogenicity of pesticides. In this paper, these workers have made reference to United States Commission on Pesticides and their relationships to environmental health, which examined data on over hundred pesticidal chemicals and concluded that at least somewhere tumorigenic and that few could be carcinogenic. Skult (1971) studied the growth and cell population kinetics of barley grown in media containing DDT. This treatment reduces the growth of main axis of the primary roots by lowering percentage of proliferating meristematic cells. The treatment also decreases the leaf growth and is toxic to root tip. Alan and Kasatiya (1974) have reported the induction of chromosome damage by the organic phosphate pesticide in Chinese hamster cells. Baquar and Khan (1971) reported the effect of γ-hexachlorocyclohexane (HCH) on the mitotic cells of pea. This compound induced binucleate and multinucleate cells and resulted in the blockage of spindle formation leading to polyploidy. Singh et al. (1973) have reported meiotic anomalies by the insecticides, endrin, cythion, ambithion, dimocran, citrolane, thimet, AC 92100, furadan and dixytron. These insecticides induced laggards, bridges, tripolar division, unequal separation and reduced chiasma frequency.
Angulo and Figueres (1969) reported the C-mitotic action of p-chloromercuribenzoate. Bennett (1971) reported the reduction of the ratio of chromosome volume and root tip cells by the fungicide ethirimol. This fungicide also reduced chiasma frequency. Levan (1971) reported C-tumor and C-mitosis induced by the hexyl-mercury-bromide. Ahmed and Grant (1972) studied the effects of mercurial fungicide panogen-15 on Tradescantia and Vicia faba root tips. This fungicide induces severe chromosomal abnormalities similar to colchicine. Vasileos and Dmitrienko (1972) reported the cytological effects of copper sulphate which is an important constituent of such famous fungicide Bordeaux mixture. Copper sulphate increases the number of pathological forms of mitosis and appears to be mutagenic. The systemic fungicide benomyl exhibits cytokinin like activity and affects the physiology of plants (Skene, 1972). Bielecki (1974) reported mitotic inhibition and polyploidy by phenyl mercury acetate. Thomas (1974) investigated the cytokinin like properties of benzimidazole-derived fungicides. These compounds produce a large proportion of abnormal mitosis. Mohan (1975) reported the antimitotic effects of the fungicides plantvax and vitavax. Richmond and Phillips (1975) have investigated the effects of benomyl and carbendazim on mitosis in hyphae of Botrytis and roots of
*Allium cepa*. In both these cases these fungicides act as mitotic poisons and induce chromosomal abnormalities. In a recent paper, Bridges (1975) has reviewed the mutagenicity of captan and related fungicides. The paper reviews such effects in bacteria, lower eukaryotic cells, higher eukaryotic cells, cytotoxicity effects on DNA metabolism, induction of chromosomal aberrations and evolution of possible mutagenic and carcinogenic hazard to man.

1-3-d Antibiotics:

Hawthorne and Wilson (1952) studied the cytological effects of the antibiotic actidione. The effects of this antibiotic are similar to those of salts of nucleic acids and superficially similar to those of colchicine. Actidione treatments show a significant increase in the frequency of various types of reductional and segregational figures. Sharma and Bhattacharyya (1967) have studied the effects of some selected antibiotic on chromosomes. Benbadis et al. (1971) studied the cytological action of chloramphenicol, cycloheximide and tubulosine on *Allium sativum* root meristems. These antibiotics caused a prolongation of $G_2$ phase of the cell cycle, which may be related to an inhibition of protein synthesis. Koop (1975) reported the action of actinomycin D, puromycin, cycloheximide, chloramphenicol and rifampicin.
on gamete formation in *Acetabularia mediterranea*. These antibiotics result in a very high frequency of cytological malformations. Bompong (1971, 1972a, 1972b and 1973) investigated the cytological effects of daunomycin and mitomycin-C. Both of these antibiotics cause chromatid and subchromatid aberrations in mitotic and meiotic chromosomes. Richard and Phillips (1973) reported the effects of griseofulvin on root tip cells. This antibiotic inhibits the cell plate formation, increases the frequency of binucleate cells and induces laggards and bridges. The induced bridges persisted up to telophase. Recently, Shah (1975) studied the effects of puromycin, chloramphenicol, actinomycin and mitomycin on cell cycles and chromosomes. According to this report chloramphenicol and puromycin inhibited protein synthesis, DNA synthesis and histone and DNA interactions. Actinomycin-D inhibited intracellular RNA, while mitomycin-C inhibited cell division. In another study Sharma and Gupta (1975) reported mitomycin-C induced chromosomal aberrations in leucocyte cultures of the Indian muntjak, *Muntiacus muntjak*, and in spleen cultures of arrow-tailed flying squirrel, *Hylotes a. alboniger*.

1-3-e Hormones:

Olszewska (1959) investigated the influence of kinetin on
mitosis. Kinetin prolongs metaphase and shortens anaphase and early telophase and significantly inhibits cell plate formation. Kallistratos (1959) studied the cytological effects of thiocolchicine, convallamarin and steroid hormones. These chemicals resulted in multinucleate cells, and tripolar and polyploid cells. Maslowski et al. (1971) reported the increase of RNA and decrease of DNA by gibberellin in maize seedlings. Kinetin and indole acetic acid induce the shortening of interphase period in the meristematic cells of onion. By using the synchronous binucleate cell population, induced by 0.1% caffeine, the interphase shortening was measured directly (Gonzalez-Fernandez et al., 1972). Bychkova and Butenko (1972) studied the action of auxin and kinetin on the mitotic cycle of a synchronized population of Fanax ginseng cells. The hormones cause the inhibition of mitotic cycle. Murin (1974) reported the acceleration of mitotic cycle by gibberellic acid. In a recent study Ved Prakash, Tyagi and Singh (1974) studied the action of zeatin, 2-furfuryl-aminopurine and 3-5-cyclic AMP on mitotic activity, and on root and shoot growth. These hormones retarded root development and stimulated shoot growth. Chromosomes, however, remain unaffected.

1-3-6 Organic and inorganic chemicals:

Abundant literature is available in this area. This
section, however, reviews some of the important contributions, from agricultural point of view, made from 1970 onwards. Diosamidze (1971) studied the cytogenetic effects of nitrosomethyl urea. This chemical decreases the rate of cell division and induces chromatid aberrations. Deysson (1971) reported synchronization of cell division in *Allium sativum* by hydroxyurea. The synchronization was followed by significant acceleration of the cell cycle. Various workers have reported the cytological and chromosomal effects of NMU (*N*-nitroso-*N*-methylurea) and NEU (*N*-nitroso-*N*-ethylurea) (Kucera, 1972; Abramov, Serebryanyi and Zog, 1972; Egamberdiev and Khazova, 1973; and Shamaeva and Garina, 1975). These compounds induce chromatid lesions, inhibit the mitotic activity, induce chromosomal aberrations and are mutagenic. Kalia and Singh (1973) reported the effects of NMU (*N*-methyl-*N*-nitroso-urethane) and MN3 (*N*-methyl-*N*-nitrosoguanidine) on the chromosome structure and mitotic activity under different temperature and pH conditions. These compounds are mutagenic and induce a wide-spectrum of chromosomal aberrations. Soifer, Telemis and Turbin (1973) reported the inhibition of DNA synthesis by hydroxyurea in pea seedling root cells.

Corman (1947), Goodwin and Taves (1950), and Sharma (1968) studied the effects of coumarin, coumarin derivatives and parasorbic acid. Coumarin produces a disruption of the
metaphase typical of many benzene derivatives, for example, suppression of the spindle, splitting and shortening of the chromosomes and retarded division of the centromere. The resultant polyploid nuclei and binucleate cells resumed division when the roots were returned to water. The effects of coumarin derivatives have been classified (Goodwin and Taves, 1950) into four groups—

(i) those which are very inhibitory and show depressing effects upon root growth, (ii) those which produce smaller inhibitions, (iii) those which produce inhibition from which the roots recover, and (iv) those which are practically ineffective.

Bonaly (1971) and Stova (1971) studied the effects of maleic hydrazide on the cell cycle and chromosomes. Maleic hydrazide induces an irreversible block at the level of $S_1$ phase and at the beginning of $S$ phase. The chemical also induces chromosomal aberration. Maleic hydrazide is used in agriculture as a side branch inhibitor, particularly in tobacco crops (Handler, 1970). Kaul (1972) reported prophase inhibition, induction of polyploidy, bridges, micronuclei, pycnotic masses and fragments by ISDA (1-isoputyl, 2-trans, 4 trans, decadienamide).

Even such universally present chemicals, like nucleic acids and amino acids, produce cytological effects. Sharma and Sen (1954) have reported induction of division in differentiated cells and also somatic reduction by the nucleic
acids. Konstantinov et al. (1970) reported the induction of mitotic disorders by the amino acids alanine, valine, leucine, threonine, asparagine, lysine-HCl, arginine-HCl, histidine-HCl, methionine, tryptophan. These amino acids caused asynchrony in the polar movement at anaphase, induced the formation of micronuclei at telophase and resulted in distribution disorders at metaphase.

Hakeem and Shehab (1970), and Bale and Hart (1973) investigated the cytogenetic effects of sodium fluoride. This chemical inhibited germination and shoot sprouting and induced chromosome despiralization and breaks, anaphase bridges, partial spindle disturbances, partial C-mitosis and micronuclei. Sodium fluoride in combination with dimethyl sulfoxide produced more severe chromosomal aberrations. Strogonov et al. (1973) have reported the induction of polyploidy, apparently by a selection process, by sodium chloride. Sodium chloride concentrations at more than 1% level proved to be lethal. Kostal (1971 and 1973) investigated the effects of copper on mitosis and roots of Vicia faba. Copper sulphate was found to be toxic to bean roots and considerably inhibited their growth. This chemical also strongly inhibited mitosis. Leonard and Gerber (1973) and Sekerka and Bobak (1974) reported the cytogenetic effects of lead. Lead produces chromosomal aberrations and disturbances of dividing figures during karyokinesis. Lead also damages nucleus, mitochondria, golgi apparatus.
endoplasmic reticulum and proplastids. In a recent paper, Mukherji and Maitra (1976) have reported complete inhibition of root growth and germination in rice by lead acetate. This compound also resulted in a drastic reduction in total chlorophyll production and relative proportion of chlorophylls a and b. Lead also disturbed mitosis and produced concentration dependant abnormalities like chromosome contraction, laggards, pycnosis, bridges and fragmentation.

1-3-g Fertilizers:

Excepting few recent reports, the work on the cytogenetic effects of various fertilizers is much less in comparison to investigations on other agricultural input chemicals. Galinsky (1949) reported the cytological effects of three phosphorus compounds (i) \( \text{Na}_2\text{HPO}_4 \), (ii) \( \text{NaHPO}_4 \), and (iii) \( \text{K}_2\text{HPO}_4 \). These phosphates produce a number of mitotic variations and abnormalities like delayed breakdown of nuclear membrane, upset of the spindle mechanism and abnormal chromosome development and distribution. In a relatively recent study, Bennett and Rees (1970) have reported the concentration dependent increase in chiasma frequency in rye by the mineral phosphate. Dement'eva and Sturua (1970) reported the fungicidal activity of the several fertilizers applied during the growth season of apple trees and
gooseberries. Nassar and Sahligy (1974) reported the induction of chromosome stickiness and spindle inhibition, and production of polyploid and multinucleate cells by the fermented blood fertilizer in spinach and beets.

1-4 The present work:

The present study, which undertakes investigations on the cytological and mitotic effects of the common fertilizers, namely, urea, muriate of potash, superphosphate and 'Gromor' (28-28-0) fills an important gap in the knowledge-growth in the area dealing with the cytogenetic effects of various crop-land input chemicals. The parameters selected for this study are as follows:

(i) Root elongation
(ii) Root number
(iii) Nucleus/cytoplasm area ratio
(iv) Somatic mitosis index
(v) Somatic mitosis chromosomal abnormalities
(vi) Meiotic mitosis chromosomal abnormalities.

The term 'mitosis' is used in its broadest sense. Wilson and Morrison (1966) classify mitosis into two common forms of cell division, namely somatic and meiotic. The former is, in general, the process which gives rise to new cells in the growing regions of an organism and maintains continuity of
chromosome number and type, while the latter gives rise to sexual gametes. This study, however, excludes the third form of mitotic activity called as endomitosis, which involves progressive duplication of the chromosomes without cell division.

The experimental design and specific experimental layouts, as adopted in this study, are described in detail in Chapter 2.
Figure 10: A hypothetical input-output model of an agricultural system
FIG. 10 A HYPOTHETICAL INPUT-OUTPUT MODEL OF MODERN AGRICULTURE