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5.1 Seed Germination and Morphological Studies:

Being a member of dinitroaniline group of herbicides, pendimethalin is considered as a selective pre-emergence herbicide. It usually prevents weeds emerging through the soil surface. This might imply that it affects the germination process of weed seeds. In the present study, pendimethalin was found to be toxic to the germination of both soybean seeds (Glycine max L. Merrill) variety KHSB-2 and maize grains (Zea mays L.) variety NAC-6002. It decreased the germination percentage in both the plant species. The percent decrease was moderate at lower concentration and severe at higher concentration in both the species but severity was higher in maize compared to soybean.

Seed germination is a complicated process which involves four phases namely imbibition phase, activation phase, mitotic phase and radicle protrudence phase (Evanari, 1957). During the process of imbibition of water, enzyme activity and rate of respiration increases in the cotyledon and endosperm of germinating seeds which results in degradation of the food reserves – namely proteins, lipids and carbohydrates (Mayer and Poljakoff-Mayber, 1963; Ching, 1972). Germination process involves mobilization and utilization of food and energy reserves from cotyledons of soybean and endosperm of maize for which it requires activation of hydrolytic enzymes, degradation of synthesized and stored food, translocation of insoluble foods, soluble food reserves and mobilization of nutrients. Hydrolytic enzymes like amylase, proteases, phosphatases, lipases etc., are known to be produced during germination (Briggs, 1963). Each of these enzymes degrade the large molecules into smaller molecules and these hydrolytic enzymes are under the control of gibberellin (Paleg, 1960). The degradation, translocation and mobilization of seed reserve are essential for synthetic reactions to facilitate the required growth and development (Norman, 1963; Amen, 1968).
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The results of the present study on the effects of pendimethalin on germination are in conformity with the observations of Nehru et. al., (1995) and Kumar et. al., (1997). Nehru et. al., (1995) reported the germination percentage of 80.7, 70.3, and 58.4 at 3, 5 and 10ppm concentrations of pendimethalin in mung bean. Similarly, Kumar et. al., (1997) have reported the inhibition of germination of maize cultivar-Suwan soaked in 5, 25 and 50ppm of pendimethalin. On the contrary many other workers have reported that the germination process is not directly inhibited by other dinitroaniline herbicides (Feeny, 1966; Schultz, 1967; Hassawy and Hamilton, 1971; Lignowshi, 1970). A significant number of preemergence herbicides, particularly acetamides inhibit the metabolic processes which are vital for seed germination (Rao, 2000).

The present investigation is in line with the findings of many researchers. Penner (1968) reported that the amount of amylase activity in the first two days of germination of barley seeds was prevented by the presence of herbicides endothal, bromoxynil, dichlorobenyl and amiben and it was not overcome by the simultaneous addition of gibberellic acid. He also reported that squash and other seeds which contain low level of carbohydrate reserves were tolerant to herbicides amiben and bromoxynil, since they were independent of herbicide-inhibited carbohydrate degradation for their energy reserves and carbon sources for anabolic processes. Similarly, Mann et. al., (1967) and Delvin and Cunningham (1970) reported inhibition of germination by various herbicides like carbamates, chlorpropham, barban, trifluralin and propachlor in grass and barley by blocking the mobilization of seed reserves due to decreased activity of hydrolytic enzymes. Dhillon and Anderson (1972), found that storage proteins were not digested in squash cotyledons even after 9 days of seedling emergence in the presence of propachlor. Rao (1974) and Rao and Duke (1974) reported that inhibition of protease and α-amylase by alachlor was prevented by GA₃. They suggested that the
herbicides may be acting as repressors of gene action preventing the normal expression and the hormonal effect of GA through the synthesis of DNA-dependent RNA. Hence, it can be suggested that most of the pre-emergent herbicides may directly or indirectly have inhibitory effect on the hydrolytic enzymes which are necessary for metabolic process of seed germination. In the present study also, inhibitory effects of pendimethalin on α-amylase activity in cotyledons were observed. High starch content in the cotyledons of developing seedlings (treated) were observed, which may be the result of decreased rate of degradation of seed reserve.

The morphological observations of six-day and fifteen-day old seedlings revealed the adverse effects of herbicide pendimethalin on early growth of both soybean and maize seedlings. There was a general reduction in seedling growth of soybean and maize which was evident by decrease in the length of root and shoot. Roots are the major site of pendimethalin toxicity as it was evident by bulged, stubby and stunted nature of roots in both soybean and maize. In soybean, the secondary root was totally inhibited even in fifteen-day old seedlings and in maize, there was a greater reduction in number of adventitious roots. The shoot growth was also severely retarded with small chlorotic leaves between the cotyledons in soybean and in the leaves of maize and tip of the axis was also chlorotic in the 15 day old treated seedlings.

The growth regulators are involved in the processes of plant growth which is accompanied by cell division, enlargement, elongation and cell differentiation in plants. The herbicides can affect the plant tissue in different ways. It may block the synthesis of various growth regulators, thus causing abnormality in the plant system (Cartwright, 1976). It may directly interfere with the division, enlargement and differentiation of cell or it may impair the functioning of various tissues of roots, leaves or vascular system on which the growth regions depend.
The reports of Schultz et al., (1968) revealed that there is direct or indirect inhibitory effect of dinitroaniline group of herbicide (trifluralin) on the processes necessary for cell elongation in corn, such as protein or RNA synthesis. They suggested that trifluralin has an inhibitory effect on RNA, DNA and protein which would results in reduced tissue growth. In addition, they also suggested that the inhibitory effect of trifluralin can be overcome by exogeneous application of IAA. Similarly Vaughn et al., (1987) reported that inhibition of seedling growth of R biotype (Resistant) of Eleusine indica at high herbicidal (dinitroaniline) concentration may be due to the secondary inhibitory effects of the compounds and not to the effects on microtubules per se. Further the decreased seedling vigour was correlated to a non-tublin related effect of herbicide. Vaughn et al., (1987) also suggested that a “direct change (genetic) in the primary structure of tublin or indirect change in tublin structure (post-transalational modification) may be responsible for the mechanism of resistance in the R biotype of Eleusine” which is, in effect a summary on the mode of action.

From the present investigation, the inhibition of mobilization and utilization of seed reserves by pendimethalin is evident by the decrease in fresh weight and increase in the dry weight of the treated seedlings over control. Further, it has adversely affected the seedling vigour, tolerance index and percent phytotoxicity of treated seedlings. These observations indicate that the rate of degradation of seed reserve may be adversely affected by herbicide directly or indirectly. In addition, the rate of cell division and cell elongation was severely affected, which may be due to the decreased seedling vigour (Vaughn, et. al., 1987), tolerance index and increased phytotoxicity.

The present study also showed that the herbicide pendimethalin inhibits the development of lateral roots, increases the radial expansion and produced stubby and stunted nature of roots near the root tips of soybean and in maize. Bayer et. al.,
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(1967) have reported that the herbicide trifluralin (Dinitroaniline group) inhibited the lateral root and increased the radial expansion of primary root in the region of elongation in Gossypium hirsutus, Carthamus tinctorius, Echinochola crusgalli and Allium cepa. They opined that enlargement of cells in the region of elongation may be due to failure of cell division and elongation. Hacskayla and Amato (1968) observed the stages of mitosis without organized cellular division, multinucleate condition, lack of cell plate formation and unequal nuclear fragments in corn and cotton root tips treated with trifluralin. Norton et. al., (1970) in Carya illinoensis and Schultz et. al., (1968) in corn has reported stunted roots, lateral root inhibition and bulbous root tips by trifluralin treatment.

Root system is the centre of synthesis of major known hormones which control plant growth, cell division and cell elongation (Torrey, 1976; Stoddart and Veins, 1980). Thus root system is an important region for all physiological activities in the plant body. Herbicides may directly affect the growth of the shoot and root by inhibition of cell division or they may impair the hormonal balance leading to the abnormal growth and they may also impair the water and mineral uptake, the transportation of organic substances to plant system (Sweeney and Marsh, 1971; Torrey, 1976; Levitt, 1980; Morejohn, et. al., 1987).

The findings on the germination and morphological studies revealed that pendimethalin inhibited seed germination and seedling growth of both crop plants soybean and maize. The root system was more sensitive to the herbicide than the shoot in maize as well as in soybean. The dry weight of treated set was higher than control in soybean and maize. These results indicate that pendimethalin may have adverse effect on the biosynthesis of GA3 which in turn affect the activities of hydrolytic enzymes during germination (Briggs, 1963; Paleg, 1960) and it may also interfere with the cell division and cell elongation (Cartwright, 1976).
5.2 PHYSIOLOGICAL STUDIES:

The inhibition of seed germination and reduced growth of both soybean and maize seedlings indicate that pendimethalin might cause impairment of specific physiological or biochemical processes. The slow degradation of protein in cotyledon and endosperm of treated soybean and maize seedlings and significant decrease of protein content in treated shoot-root axis of soybean and embryonic axis of maize over control indicates that pendimethalin interferes in synthesis of enzymes directly or indirectly, which are essential for degradation and synthesis of protein in the seedling during germination and its early growth. Hence, pendimethalin affects the rate of degradation of storage proteins in cotyledon of soybean and endosperm of maize and also affects the rate of synthesis of protein in shoot-root axis and embryonic axis in both the crops.

During seed germination and seedling growth, the seed reserve gets hydrolyzed and a change in the cellular and organelle constituents such as proteins, lipids and carbohydrates takes place. However, the rate of change varies from crop to crop and species to species (Ching, 1972; Ashton, 1976 and Osborne, 1962). Protein is the major storage reserve. The storage proteins are hydrolyzed by the proteolytic enzymes into amino acids. These amino acids are utilized by the developing seedlings for the synthesis of various enzymes and structural proteins. In the present investigation, the observed high protein content in the cotyledon of soybean and endosperm of maize may be attributed to the inhibition of proteolytic activity of these enzymes (Ashton, et al., 1968; Penner, 1970). Ashton et. al., (1968) studied the proteolytic activity of some 26 herbicides in an attempt to correlate this activity with herbicidal effect through inhibition of seed germination in squash seedlings. They have demonstrated that $1.5 \times 10^{-6}$ M trifluralin inhibited the development of proteolytic activity by 41% in the cotyledons of 3- day old squash seedlings. Further, Penner (1970) has also showed inhibited development of
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Phytase activity during germination at $10^{-5}$ M trifluralin concentration in barley seedlings, squash cotyledon and maize embryo by 12 to 24%. The decreased rate of protein degradation in both the species may be correlated with the inhibition of proteolytic enzymes. The observed slow degradation of proteins in the cotyledons of soybean and endosperm of maize seedlings may be due to the decrease in the rate of protein synthesis in the shoot-root axis of soybean and embryonic axis of maize. These results are in accordance with the observation of Mann *et. al.*, (1965) and Moreland *et. al.*, (1969). Moreland *et. al.*, (1969) have studied the effects of some 22 herbicides on the synthesis of RNA and protein in excised maize mesocotyl and soybean hypocotyls section. The assay measured ATP and orotate incorporation into RNA and leucine incorporation into protein. They found little effect on $^{14}$C-leucine incorporation into protein of soybean hypocotyl section. Likewise, Mann *et. al.*, (1965) used 23 herbicides in tests on the incorporation of leucine into protein. They observed little effect of trifluralin on $^{14}$C-leucine incorporation into barley coleoptile sections or hemp sesbania (*sesbania exaltata*) hypocotyl sections. Further, Tsay and Ashton (1971) showed inhibition of development of dipeptidase activity in squash cotyledon by 16% at $1.5 \times 10^{-6}$ trifluralin after 4 days treatment. Many herbicides forms complexes with amino acids, carbohydrates and other cellular constituent which may contribute to the overall biochemical responses to the herbicide. The lower level of protein content in both the species may be attributed to the inhibition of enzyme synthesis by inhibitors of protein synthesis.

Carbohydrates are important components of storage and structural materials in plants. They exist as free sugars and polysaccharides (Hodge and Hofreiter, 1962). They are the energy rich sources and are essential for germination of seeds. Germinating seeds are dependent on starch for their energy supply which is degraded into soluble forms by $\alpha$-amylase enzyme. Further, phosphorylases and $\beta$-amylase enzymes are also responsible for the conversion of soluble forms into free
sugars (Dunn, 1974; Juliano and Varner, 1968). In the present study, the total carbohydrates, starch and reducing sugar content were higher over control in cotyledons of soybean and endosperm of maize in all the treatments. Conversely, they were found decreased in shoot-root axis and embryonic axis in all the treatments over control. Hence, it can be inferred that pendimethalin interferes with the metabolism of carbohydrates in germinating seeds. It may impair the rate of degradation of carbohydrates in cotyledons and endosperm. Similarly its synthetic activity was impaired in shoot-root axis and embryonic axis of both the crop species under investigation.

Various researchers have observed the inhibition of degradation of starch and sugars in herbicide treated seedlings. Duke et al., (1985) reported that the accumulation of starch in etioplast is due to the blockage of sugar utilization during metabolism by trifluralin. Duke and Biswas (1967) observed an increase in the carbohydrate content of sweet potato and peanuts treated with trifluralin at 5 and 10ppm concentrations. Kust and Struckmeyer (1971) reported the accumulation of starch in the xylem elements and nodules of soybean roots when seeds were treated with trifluralin. In the present study starch is the predominant carbohydrate content which is higher in the cotyledons of soybean and endosperm of maize which may be due to the slower breakdown as a result of lower amylase activity (Jones and Foy, 1971). The low value of total sugar compared to starch in the treated seedlings clearly demonstrates the possibility of high rate of utilization of soluble sugars by the seedlings in response to stress induced by pendimethalin.

Dalvi et al., (1972) reported similar toxic effects of menazone, disulfoton and GS-14254 on wheat and mung bean. They found a decrease in reducing sugars and free amino acids content in treated seeds and correlated this to decrease in starch degradation in pesticide treated seeds. Chopra and Nandra (1969) have investigated the effects of thiometon on Brassica campestris and observed a
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decrease in both reducing and non-reducing sugars in treated plants. They have attributed the decrease in reducing sugars to the decreased activity of the enzyme lipase. This in turn causes decreased breakdown of fat and its conversion to carbohydrates resulting in the reduced production of reducing and non-reducing sugars. Felipe et. al., (1986) observed the effect of isoproturon on photosynthetic apparatus. In another study, Felipe et. al., (1988) reported the decline in RuBP carboxylase activity and chlorophyll in Lolium temulentum after treatment with isoproturon. Later Dhawan et. al., (1992) also found the degeneration of chlorophyll in Phalaris minor sprayed with isoproturon. They have concluded that the decrease in reducing sugars and protein may be due to a diminished supply of NADH and ATP because of degeneration of photosynthetic pigments. Since most of the enzymes appear to be synthesized de novo and are induced by the hormones gibberellic acid or cytokinins, it is possible that pendimethalin may interfere with the action, development or transport of these hormones. The herbicidal action on the degradation of reserve materials and on the control of the various related processes during germination may be one of the factors responsible for susceptibility of seedlings to the herbicide.

The phenol content was found decreased in cotyledon of soybean and endosperm of maize, whereas in shoot-root axis and embryonic axis total phenol content was increased over controls. But the increase was significant only in higher concentrations of pendimethalin.

Phenolic compounds, a diverse group of plant secondary metabolites play an important role in the regulation of plant growth and development. Changes in phenolic compounds are very characteristic in plants. It plays an important role in determining the resistance or susceptibility of plant to any type of environmental stress or toxicity. The resistant varieties have been found to contain more phenol than susceptible ones (Raghunathan, et. al., 1958; Rubin and Aksenova, 1957). An
increase in the phenol metabolism following infection has been reported by various workers (Chattopadhyay and Ber, 1980; Thind, et. al., 1977). Chattopadhyay and Ber, (1980) have attributed the gradual decrease in the quantity of phenol in the resistance variety to greater polyphenol oxidase activity. The polyphenol oxidase is responsible for the conversion of phenol to quinine. Vijayabhaskar et. al., (2001) observed that phenol content increased with increase in potassium application, while decreased with increase in nitrogen application. The increased phenol content in potassium applied plant might be due to the effect of potassium (Ramaswamy and Prasad, 1974).

Six day and fifteen day old pendimethalin treated seedlings were either yellow or pale green in colour in both soybean and maize. The studies on the chlorophyll-a, chlorophyll-b and total chlorophyll contents in soybean and maize seedlings treated with pendimethalin revealed the adverse effects of herbicide on chlorophyll content. Moreland et. al., (1972a) suggested that 2,6 dinitroaniline herbicides inhibit the oxidative and photophosphorylation in mitochondria and chloroplast respectively. They found that both photoreduction and coupled photophosphorylation with water were inhibited in isolated chloroplast of spinach. Moreland et. al., (1972b) have also demonstrated the interference of 2,6-dinitroaniline herbicide with photosynthesis and respiration both in vitro and in vivo. At elevated concentration trifluralin inhibits photosynthetic electron flow. Their studies revealed that 2, 6-dinitroaniline interferes with ATP production leading to the phytotoxic action. The chlorotic effects of photosystem II inhibitor occurs slowly after root uptake. Under these conditions membrane lipid peroxidation occurs rapidly, resulting in necrosis and desiccation (Devine, et. al., 1993). Inhibition of photosynthesis and photosynthetic electron transport by herbicides leads to destruction of the existing chloroplast pigment (Rao, 2000). On an ultrastructural level, chloroplast swelling and membrane rupture can be observed
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after 5-10 hrs depending on the irradiance level. At the highest irradiance levels, membrane rupture occurs prior to any other visible effects indicating the high sensitivity of membrane and chloroplast envelopes in particular to damage by oxidants (Pallett and Dodge, 1980).

Many herbicides cause either white or yellow chlorosis of leaves as a consequence of total or partial absence of the normal chloroplast pigments (Duke, *et. al.*, 1985; Duke and Kenyon, 1986; Vencill, *et. al.*, 1989). Nehru *et. al.*, (1999) reported a decrease in chlorophyll content in mung bean by three herbicides including pendimethalin at 3, 5, 10ppm. Chlorosis may result from the inhibition of pigment biosynthesis or from destruction of the existing pigments. Pigment biosynthesis inhibitors cause chlorosis only in newly developed leaves and they are most effective with pre-emergence herbicides. Chlorosis of seedlings occurs due to the inhibition of terpenoid biosynthesis. Chlorosis of seedlings may also results due to the subnormal level of iron and magnesium content in the plant body. The inhibition of acetyl Co-A carboxylase, alloxydin and sethoxydium indirectly inhibits chloroplast pigment biosynthesis and cause leaf chlorosis (Asare-Boamah and Fletcher, 1983; Ikai *et. al.*, 1982; Lichtenthaler *et. al.*, 1987). In addition, inhibitors of photosynthetic electron transport may produce chlorotic effects and disturbs the existing pigment and photosynthetic activity. It may be concluded that the reduction in chlorophyll content by pendimethalin may be due to inhibition of pigment biosynthesis or from destruction of the existing pigments.

Enzymes play an important role in metabolism. They are exceedingly efficient and very specific in terms of nature of reaction catalyzed and the substrates utilized. The synthesis and final concentration of enzymes is under genetic control and is greatly influenced by very small molecules of substrate. These cellular catalysts control the formation of biochemical intermediates essential to all physiological functions. Hence, change in enzyme levels is one of the fundamental
steps to assess the effects of toxicants. The decreased activities of various enzymes in herbicide treated seedlings have been reported by various researchers in various crops.

In the present study, $\alpha$-amylase activity was found decreased in both parts of soybean and maize seedlings with increasing concentrations of pendimethalin over control. This study clearly indicates the adverse affects of pendimethalin on $\alpha$-amylase activity. It also clearly substantiate reduced rate of degradation of total carbohydrates and starch in the treated seedlings. Penner (1968) in his findings revealed that the production of amylase in the germinating seeds is under the control of either embryo or by exogenous application of gibberellic acid. Chrispeels and Varner (1967) and Jones and Foy (1971) revealed that gibberellic acid not only triggers the synthesis of $\alpha$-amylase, it is also required continuously during the enzyme formation. The inhibitor of protein and RNA synthesis inhibits the formation of ribonuclease leading to inhibition of synthesis of $\alpha$-amylase. Robles and Mercado (1971) also reported the inhibition of $\alpha$-amylase activity by trifluralin which impaired the degradation and mobilization of seed reserves. Since gibberellic acid also controls the metabolism of ribonucleic acid during the production of hydrolytic enzymes and protein synthesis, the blockage of either RNA or protein synthesis may result in the inhibition of $\alpha$-amylase production (Chrispeels and Varner, 1967; Chandra and Varner, 1965; Moreland, et. al., 1969). In the present study, the $\alpha$-amylase activity was inhibited in treated sets of soybean and maize, which may be due to the interference of pendimethalin in the metabolism of phytohormones such as gibberellic acid.

The enzymes catalase, peroxidases and polyphenoloxidase also play an important role in plant metabolism. They protect the plant body from the harmful effects of hydrogen peroxide and are also involved in the scavenging of active oxygen radicals. Catalase and peroxidase play a considerable role in the growth
processes of plants by controlling the auxin catabolism and affecting the level of auxin like substances (Dekock, et. al., 1960; Pilet and Gaspar, 1968). Both these enzymes have been found to vary in plants treated with growth retarding chemicals. They catalyze the dehydrogenation of organic compounds in higher plants. Catalase oxidizes the toxic substances formed during the development which otherwise are lethal to plant. Peroxidase uses the hydrogen peroxide as substrate and catalyzes the oxidation of many mono and diphenols and aromatic amines to quinines (Bonner, 1950). Polyphenoloxidase is known to oxidise a very wide range of substrates including monophenols, triphenols, ascorbic acid and p-diphenol (Mayer and Harel, 1979). The elements potassium, magnesium and iron are responsible for the production of catalase, peroxidase and polyphenol oxidase (Marsh, et. al., 1963; Nanawati, et. al., 1973). Hence, these three enzymes are considered as indicators of physiological stress (Levitt, 1972).

The observed increased activity of enzymes catalase, peroxidase and polyphenol oxidase in the treated set over control shows that the pendimethalin stimulates the synthesis of these antioxidant enzymes despite quantitative differences under varying concentrations. Seed treatments initiate higher mitochondrial activity leading to formation of more high energy compounds and this possibly helped the plants to resist adverse environmental conditions (Henckel, 1964). The favourable effects of seed soaking in water and other chemicals might be due to their quenching effect in counteracting the formation of free radicals (Basu, et. al., 1984). The activity of antioxidant enzymes enhanced in order to reduce membrane damage against active oxygen radicals. Enhanced activity of catalase was reported to be essential for the survival of halophyte *Halimione portulacoides* (Kalir and Poljakoff-Mayber, 1981). Enhanced activity of catalase may be due to toxic substance formed during the development of plant. Increase in peroxidase activity in salt tolerant varieties of *Pisum sativum* was observed by
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Olmos *et. al.*, (1994) and Singh *et. al.*, (2001). This increased peroxidase activity may be due to increased enzyme synthesis which is useful for adaptation of membrane lipids (Kalir, *et. al.*, 1984). Increased polyphenol oxidase activity may be due to oxidation and degradation of the toxic substances such as phenolic compounds which are accumulated during environmental stress. Both peroxidase and polyphenol oxidase activity increases in the species subjected to various environmental stresses (Subhashini and Reddy, 1990).

The physiological studies demonstrate that the adverse effects of pendimethalin on the plant metabolism may be due to the inhibition caused by the herbicide which in turn affects the activities of various enzymes. Since all the enzymes appear to be synthesized *de nova* and are induced by the hormones gibberellic acid or cytokinins, depending on the species, it is possible that pendimethalin interferes with the action, development or transport of hormones. The observed reduction in the total protein, total carbohydrates, total starch, total reducing sugar, different enzymes and chlorophyll in treated seedlings may also be due to disrupted membrane permeability, leading to leakage of ions essential for metabolic activities and also due to the interference of pendimethalin on hormones gibberellin and cytokinin.
5.3 MITOTIC AND ULTRASTRUCTURAL STUDIES

MITOIC STUDIES:

Cytogenetic studies are important for obtaining information regarding the role and effects of various toxic substances and elucidating the response of various genotypes to a particular toxicant. The mitotic processes are subjected to modifications by extraneous factors. The lengthened or shortened cell cycle by certain chemical substances is one such modification (Davidson, 1968; Sharma and Sharma, 1972). This effect may be discerned from the variation in the mitotic indices, as they are the morphological indicators of the ratio between the mitotic time and the total cell generation time (Van't Hof, 1968). Many herbicides are known to act as mitotic poisons by disturbing the mitosis in the meristematic regions (Bayer, et. al., 1967). From the present study, it is evident that pendimethalin affects the cell division in the primary root meristem of both soybean and maize. In both the crops, mitotic indices were declined with increasing concentrations indicating the toxic nature and mitotic poisoning action of pendimethalin. Various abnormalities of mitosis due to pendimethalin treatment observed in the present study are C-metaphase, polymorphic nuclei, isodiametric cells, abnormal xylem development, swollen root tips. These results are in accordance with the effects of many other dinitroaniline herbicides as reported by many workers (Hackaylo and Amato, 1968; Schultz, et. al., 1968; Bayer, et. al., 1967; Talbert, 1967; Beuret, 1980). These researchers observed the inhibition of root growth which is accompanied by an increase in diameter or swelling of the root near the root tip or in the meristematic regions as well as inhibition of lateral root or secondary root development. Cytological studies with dinitroaniline treated roots indicate that mitosis is inhibited due to disorganized nuclear division and these dinitroaniline herbicide have been classified as mitotic poisons (Parta and Soper, 1977).
Many dinitroaniline herbicides cause mitotic and ultrastructural effects similar to those caused by colchicines, but are sometimes found much more effective than colchicine (Devine, et. al., 1993). They inhibit the growth of root tips and cause root tip swelling that is distinguishable from that induced by colchicine (Jackson and Stetler, 1973, Nishmoto and Warren, 1971; Wang, et. al., 1974). Arrest of cell division at prometaphase, was reported by Vaughan and Vaughan (1987), Lignowski and Scoot (1972), Armbruster et. al., (1991) and Hackaylo and Amato (1968) in some crop plants treated with herbicides. Lignowski (1970) and Lignowski and Scoot (1972) observed that onion and wheat root tips treated with trifluralin exhibited an increase in the percentage of cells in arrested metaphase and a decrease in anaphase and telophase stages three hours after treatments. Bartels and Hilton (1973) observed that trifluralin and oryzalin arrested cell division at metaphase in corn and wheat root tips with a few telophase stages in trifluralin treated roots. Genter and Burk (1968) saw numerous chromosome aggregations corresponding to the metaphase stage of mitosis in nitralin treated corn roots. The absence of cell plate and cell wall formation was also noted in trifluralin treated roots of corn, cotton (Hackaylo and Amato, 1968) and cell-wall free endosperm cells of Haemanthus katherinae (Jackson and Stetler, 1973)

According to Armbruster et. al., (1991) and Hackaylo and Amato (1968), the chromosomes were arrested at prometaphase as a result, the chromosome become thickened and shortened. Then gradually clumping of chromosome occurs to form micronuclei which some time fuse to form large, amoeboid nuclei and also polyploid nuclei. Multinucleate cells were common in such cells. At higher concentration, high degree of vacuolation and degeneration occurs resulting in the subsequent loss of meristematic activity as reported for many other dinitroaniline herbicides (Bayer, et. al., 1968; Ennis, 1948; Devine, et. al., 1993). Formation of bi
and multi-nucleate condition of cells may be due to the lack of cell plate formation ( Vaughan and Vaughan, 1987; Schultz et. al., 1968).

The root tip cells treated with pendimethalin showed arrested mitotic stages, clumping of chromosomes and bi-nucleate to multi-nucleate conditions. In addition, the root tips showed loss of meristematic tissue or enlargement of meristematic cells. The abnormalities produced by pendimethalin in root meristem are not uniform. With reference to dinitroaniline herbicides Talbert (1967) reported that root growth inhibition in soybean was associated with cessation of cell division in the meristematic tissue. He also reported there was increase in prophase figure and no other stages of cell division detected. In addition, he found that mitotic activity was not affected in all cells. A comprehensive investigation on the morphological and histological effects of trifluralin on root development of cotton, safflower, onion and barnyard grass were conducted by Bayer et. al., (1967). They found that trifluralin disrupted the mitotic processes and exert external effect on primary root by increase in the radial expansion near the root tip. They speculate that it might be due to a differential activity of cells according to the type and stage of differentiation.

Cytological examination of the root meristem showed that the adverse effects of the herbicide pendimethalin include disrupted mitotic process, enlargement of cells, prevention of cell wall formation, and extensive replication of nuclei, hypertrophy and hyperplasia. Further, there was increase in prophase but practically no other stages of cell division detectable. Tubulin a dimer protein in the cell polymerizes to form microtubules. Microtubules form the major part of the mitotic apparatus including spindle fibers, which enable chromosomes to separate during cell division. Direct or indirect inhibitory effect on polymerization of microtubules results in arresting mitosis leading to abnormal cell with more than the normal complement of chromosomes and frequently, lobed nuclei and multinucleate
condition. In addition disorientation of microfibrils, irregularly shaped cells are the common symptoms. It is possible that pendimethalin may interfere with the microtubule formation which in turn affects the cell division and cell elongation.

**ULTRASTRUCTURAL STUDIES:**

Ultrastructural studies can often provide insight into the mechanism of action of herbicides. Relatively little is known about ultrastructural and immunocytochemical effects of chemical compounds on somatic cells and cell organelles (Bartels, 1965). The observed irregular shape, variable size, electron opaque deposits and narrowed cell wall of pendimethalin treated root tip cells of soybean and maize may be due to the abnormal pattern and orientation of cell deposition. The cell wall of treated root tip cells showed disruption and disintegration with middle lamella in certain portions and also disrupted plasma membrane. These effects may be due to the cells that are arrested in prometaphase and the formation of the nuclear membrane around the chromosome. The nucleus becomes lobed probably due to the spreading of chromosomes throughout the cytoplasm and subsequent formation of nuclear membrane around the chromosome (Vaughn and Lehnen, 1991). It is reported that no spindle and kinetochore tubulin are formed in the dinitroaniline treated cells. Further the cortical microtubules which determine the shape of the root cells in the zone of elongation by directing the deposition of cell wall compounds were also affected by this herbicide (Hess, 1987). The net results are isodiametric rather than elongate cells contributing to the swollen appearance of root tips. The abnormal cell wall deposition and orientation due to herbicide sindone treatment has been reported in onion (Lehnan and Vaughn, 1992) and wheat (Ambruster, *et. al.*, 1991). The ultrastructural studies of dithiopyr on wheat root tips cells revealed that dithiopyr leads to the extreme deposits of extraneous wall polysaccharides and loss of cell plate orientation (Ambruster, *et. al.*
Lehnen and Vaughn (1998) in their finding reported the branched cell wall with electron opaque inclusions in the cell wall, lobed nucleus and bi-nucleated condition in onion root tips treated with dithiopyr. Center and Burk (1968) after cytological examination of nitratin affected area suggested that it prevents cell wall formation and extensive replication of nuclei. Vaughn and Duke (1986) in their finding observed polymorphic nucleus resulted due to reformation of nuclear membrane as a result of arrested mitosis. The observations made in the present study are in conformity with the above findings.

The ultrastructural studies also showed that plasma membrane was not in contact with the cell wall having plasmolysed appearance in affected cell. This may be due to loss of differential permeability property of plasma membrane and cells did not take up water by osmosis as reported by Berjak and Villiers (1972c) and Normah and Chin, (1989). Baur and Bowman (1972) reported that the herbicide paraquat disrupts membrane integrity to possibly cause wilting necrosis and eventual death of foliage. Similarly Sutton and Foy (1971) reported that the herbicide diquat altered the electrostatic stability of the membrane resulting in deterioration of membrane integrity.

With increase in the pendimethalin concentration, severe damage of cells by exhibiting swollen secondary phase lysosomes and the large vacuoles appeared with degenerated organelles. The disruption of plasma membrane may cause leakage of specific ions such as potassium and sodium and also adversely affects the uptake of these ions. Loss of membrane control may lead to the activation of lysosomes by affecting the lysosomal membranes which would allow leakage of wide range of hydrolytic enzymes leading to amplification of pendimethalin damage causing general lysis of the cell. Lysosomes which must normally retain their hydrolytic enzymes probably had also undergone degenerative changes during storage which caused bursting during imbibitions of the seed. This was followed by general lysis.
of the cytoplasm and all internal organization was lost. Zweig et al. (1965) reported that diaquat generally affects the plant by their ability to form toxic free radicals by the processes of reduction and subsequent reoxidation to yield \( \text{H}_2\text{O}_2 \) and \( \text{OH} \). They also reported that it inhibits the reduction of NADP to NADPH by removing the electrons from the electron transport system of Photosystem II. The instantaneous reoxidation of bipyridilium ion to form \( \text{H}_2\text{O}_2 \) appeared to be important in the activity of diaquat and paraquat. There is evidence that free-radical lipid peroxidation may contribute to the aging process as a mechanism in the damage of cellular membrane systems viz., mitochondrial, endoplasmic reticulum and lysosomal membranes (Tappel, 1965). Berjak and Villiers (1972a, b) observed the ageing damage to the membrane system which is presumably accumulated during storage. Kalpana and Madhava Rao, (1993) also observed similar membrane damage followed by accelerated lysosomal activity in the pigeon pea when subjected to ageing treatment.

The ultrastuctural studies demonstrates that the herbicide pendimethalin may disrupt the plasma membrane resulting in leakage of ions necessary for metabolic activity and increase lipid peroxidation of membrane. The enhanced lysosomal autophagic activity may be attributed to the increased free radical formation and subsequent damage of cellular membrane system. The observed irregular shaped cell and irregular thickness of cell wall deposit may be auxin induced wall loosening or by reducing turgor pressure by direct or indirect inhibition at the metabolic level. The bi-nucleate to multi-nucleate condition may also be due to the inhibition of microtubule formation.