LITERATURE REVIEW

1.2. LITERATURE REVIEW

As the present dissertation mainly focuses on two aspects i.e. biology of *Sechium edule* and influence of plant growth regulators on growth, metabolism and yield, the review is prepared with the available literature on the biological aspect of the experimental plant as well as with that on the regulatory aspect of a few plant growth regulators, particularly growth retarding chemicals. Hence this review is dealt under two major captions : 1. Biology of chayote (*Sechium edule* Sw.) - a brief historical overview and 2. Plant growth retardants-their regulatory actions on growth, metabolism and yield.

1.2.1. : *Biology of chayote (Sechium edule Sw.) - a brief historical overview* :

As chayote is a minor vegetable crop and less known to the scientific world, hence, a brief introduction of this plant with respect to its botany, propagation method, varieties, chemical and nutritional composition are included in the present review. A sustantial volume of literature is now available with this minor vegetable crop and these are contributed by some workers in diverse fields of research (Cook, 1901; Chakrabarty, 1973, Aung, 1976; Zinsou *et al.* 1983; 1985; Zinsou and Vansuyt, 1985; Lorenzi *et al*, 1988; Aung *et al.*, 1996; DiGregorio *et al.*, 1997; Piaggesi, 1997).

1.2.1.1. : A short botanical account of the plant :

The plant is a climbing herb with extra-axillary, single or branched tendrils, monoecious. The above ground part is annual and die in winter but underground part i.e., tuberous roots, is perennial. The vegetative plant (vine) is large and grow vigorously. Angular stem of vines bear strongly tri-angled or lobed leaf with broadly cordate base having four sharp corners. Vines, longitudinally furrowed and covered sparsely with trichomes, and axillary borne male and female flowers. The male flowers borne on peduncle in clusters ranging from 20-25 flowers in each cluster. Female flowers develop solitary or in pairs, often in the same axil of the male flowers. The young fruit is mainly pubescent and with maturity it becomes spiny in some varieties. The mature fruit is compressed and pear-shaped with large single flat seed (Chakrabarty, 1973; Engles, 1983), but shape vary with varieties. The surface of the fruit is usually more or less uneven with several deep longitudinal grooves or channels.

1.2.1.2. : Propagation of the plant :

The chayote plants propagate by the entire fruit with single flat and large seed. Unlike other cucurbits having multiple seeded fruit, chayote bears single seeded fruit and shows viviparous germination. The chayote seed is cordate, flat, and is embedded centrally in the mature fruit. The seed-coat is obsolate, before germination; the seed grows in such manner that the apex of the cotyledon moves towards the base of the fruit and hypocotyl emerges from the apex and gives rise to several rootlets. In the middle of the outer faces of the cotyledons, there is a considerable area which presumably absorb the nutritional materials from the fruit and supply to the growing seedling (Cook, 1901). It is also observed that chayote propagation can be done by both vegetative and reproductive means. The storing of moisture and food in the form of tuberous root assist in carrying the young plant through periods of drought or adverse condition. After sprouting fruits send out several feet long healthy vine to make connection with the soil without the seed making possible way of existance where the ground is covered with tangled masses of vegetation.

1.2.1.3. : Varieties of Chayote :

Cook (1901) and others reported the existence of two distinct varieties of *Sechium edule*, the first with green coloured fruit and other is white. Cook (1901) found that in Porto Rico, there were five varieties of the plant and one of the white kinds being as small as any of the green variety. Two varieties grown in Madeira, the cream coloured or white variety was larger in size. Engels (1983) reported eleven varieties of chayote from Central America. And the present worker reported ten varieties of chayote from Darjeeling hills (Lama *et al.*, 1994).

1.2.1.4. : Chemical and nutritional composition :

The most ancient literature on chemical composition of chayote plant as searched by the present worker is the work of Cook (1901) who analysed the composition of edible storage tuberous roots of chayote. In 100 parts of the tuber he estimated 71% water, 20% starch, 0.20% ether soluble resinous material, 0.32% sugars, 0.43% albumen, 5.60% cellulose, 2.25% minerals. Aung *et al.*, (1976; 1990; 1991; 1992) found that the

storage roots of a light-green type of chayote contain 0.6% soluble sugars and 13.6% starch on wet weight basis. Thus the ratio of starch : sugar in the tuberous root is 23:1. Again, they found 0.3% soluble sugar and 0.7% strach in young apical shoots. Analysis of the chayote fruit flesh showed 3.3% soluble sugar and 0.2% strach; in the seed 4.2% soluble sugar and 1.9% strach was found on wet weight basis. They observed that fruit contains 15 and 25 times more soluble sugar than tuberous root and apical shoots respectively whereas strach content is very much less. Aung and his associates (1976; 1978; 1990; 1991; 1992) further recorded that the carbohydrates in chayote consist of fructose, glucose, sucrose, sorbitol, raffinose, stachyose and strach. In the vegetative shoot, the fructose content was four times and glucose content was two times greater than sucrose or sorbitol. In the male flower, 79% of the soluble sugars consisting of fructose and glucose were found in the floral disc and nectaries. In the androecium, sucrose content was 1.2 times greater than fructose or glucose. The fruitlet was found to contain six times more fructose and glucose than sucrose. In the immature fruit, the strach content was higher in the seeds (85 $\mu g.mg^{-1}$ dry wt.) than the flesh (75 $\mu g.\ mg^{-1}$ dry wt). Lama *et al.* (1994) reported that among the three chayote types growing in three different altitudinal zones of Darjeeling hills, the type growing in Mirik was superior in all respects, particularly in yield attributes. As compared to young fruits, protein and insoluble carbohydrate levels were found high in mature fruits. But soluble carbohydrates remained at low level in mature fruits. On the other hand, catalase, dehydrogenase, and α-amylase showed maximum activities in mature fruits. Flick *et al.* (1978) reported that chayote fruit contain high moisture and low nitrogen and about 0.4% crude fibre on the whole fruit. The seed of the chayote was considerably higher in all 18 amino acids than the flesh. Methionine was detectable in seed but not in the flesh. Of the total nitrogen 59.9% was protein nitrogen. The activities of differnet enzymes like ATPase, F-1,6-Dipase, G-6-Pase and G-1-Pase were observed in chayote. ATPase and G-1-Pase showed highest and lowest in seed and flesh respectively. ATP substrate introduction accelerated phosphatase activities more than Glucose-1-6-phosphate. The other activities decreased in the following order : F-1-6-diPase, α-glycerol phosphatase, G-6-Pase and phytase. Skin of the fruit showed maximum phosphatase activities than the seed.

Apart from the work of Aung, Ceccarelli and Lorenzi (1982, 1983, 1990, 1992) variously documented the endogenous hormonal regulation on chayote seed and its

germination. Ogawa (1966) reported the first existance of 'water soluble' gibberellin - like substances in developing chayote seeds. Albone *et al.* (1984) reported the presence and localization of gibberellin catabolites in the testa. It is reported that the immature fruits of chayote contained very high levels of growth regulators, particularly gibberellins and cytokinins (Lorenzi, 1988; Ceccarelli, 1992). A thorough investigation of Ceccarelli and Lorenzi (1992) on the endogenous gibberellins and cytokinins in chayote seed revealed that the endosperm tissues of the chayote seed contained maximum gibberellins than other plant parts. The distribution of gibberellins among different tissues of the seed showed different in qualitative and quantitative ways and gibberellins content of endosperm does not change qualitatively in all the cases of seeds while quantitative increase was observed in more mature seeds. It has been demonstrated that endosperm and cotyledons synthesized gibberellins simulteneously in the seed which can be directly proved by the work with cell-free systems. Endosperm and cotyledons of *Sechium edule* at different stages of seed development were found to contain three novel GA conjugates namely a glucoside of 16-17 dihydro-16-hydroxy-GA_{15}, the 3-propyl-or 3-acetayl-GA_4, the 3-propyl or 3-acetyl-GA_7. The function of these conjugates are not well understood. The *S. edule* GA glucoside is biologically active in the lettuce and rice bioassy. In GA glucoside, the aglycone resulting eventually from enzymatic hydrolysis would not bear structural characteristics assuring biological activity. Thus it seems reasonable to hypothesize that the observed biological activity would result from its further metabolism. This suggested that GA conjugates act as a transporter or storage form of *S. edule*. The endosperm of chayote contains high amount of GAs both free and conjugated *in vivo*. The lower ABA concentration in pollinated ovules than unpollinated ones suggested that the pollination accelerated the ovule growth in faster rate which caused dilution of ABA level continuously. Apart from these two hormones, gibberellins and ABA, *S. edule* seeds contain significant amount of cytokinins like zeatin, riboside, ribotide and O-glucoside. Vansuyt and Zinsou (1986) reported that the agmatine, the immediate decarboxylation product of L-arginine in floral buds and apical part of the leaves accumulated more agmatine than basal part. The accumulation of agmatine during the flowering stage considered as a good biochemical marker for cell division in chayote. Vozari-Hampe *et al.* (1992) suggested that the exudate of *S. edule* fruit contained lectin which was rich in glycine, leucine, asparagine/aspartic acid, glutamine/glutamic acid and serine residue, without detectable amount of methionine and hydroxyproline. The purified chayote lectins were non-specific towards human

erythrocytes of A,B or O groups. Besides the above mentioned chemical compositioin of chayote, MacLeod (1990) reported different volatile components of chayote. A total of 61 components were identified and four were partially characterized.

Table : 1.1 Volatile components of the chayote : (DB5 column GC-MS)

Components	$Rt_{(min)}$	Kovats index	%RA (relative abundance)	Odour
Butanedione (i.e., diacetyl)	7.43	575	0.1	Sweet, creamy, buttery.
Hexane	7.71	600	6.1	
2-methylbutan-2-ol	9.77	-	0.1	
3-methylbutan-al	10.64	649	0.7	fresh green, fragrant, chemical solvent.
Cyclohexane	11.08	677	0.2	stale green, chemical solvent.
2-methylbutanal	11.24	651	0.3	sweet, rotting fruit.
Pent-1-en-3-ol	12.33	673	0.3	green, buttery.
Pentane-2, 3-dione	13.25	681	0.2	buttery, caramel, toffee.
Pyridine	17.05	695	0.4	stale grass,chemical solvent
2,4-Dimethylhexane	18.79	-	0.1	
Toluene	19.12	765	tr.	chemical solvent.
3-methylheptane	19.44	-	0.6	
A dimethylcyclohexane	19.99	-	0.3	sap-like, fragrant.
Octane	21.83	800	0.6	oily/fatty, sweety, rancid.
Hexanal	21.94	780	1.3	green,grassy,fragrant, oily/fatty.
2-Furaldehyde/furfural	24.82	815	tr.	oily/fatty,caramel, roasted.
(E)-Hex-2-enal	26.89	832	0.1	green, grassy, beany, fragrant.
(Z)-Hex-3-en-1-ol	27.16	847	10.1	fresh cut grass,green
Hexan-1-ol	28.25	858	1.1	green, grassy, fragrant, earthy, oily.
Benzaldehyde	37.18	947	tr.	nutty, almonds.
Oct-1-en-3-ol	38.46	968	10.4	green,grassy,earthy, musty, mouldy, cucumber like.
2-Pentylfuran	39.71	983	0.2	green, hay, rubbery
(E,E)-Hepta-2, 4-dinal	41.39	989	0.1	oily/fatty,putty,green, cucumber-like

Contd...

4-iso Propyl-1 methylbenzene (i.e.-p-cymemne) Limonene	42.30	1020	tr.	fragrant
	43.24	1022	3.0	sweet,fragrant,lemongrass
Phenylacetaldehyde	44.65	1024	0.1	floral,fragrant,roses, hyacinth.
Decan-2-one	56.72	1176	0.1	fragrant,caramel.
Napthalene	57.30	1172	tr.	fragrant, camphoraceous
Benzothiazole	60.31	1202	tr.	
2-Methoxy-4-vinylphenol (i.e.,p-vinylguaiacol)	66.60		0.2	stale,musty,cooked beans,cloves.
1-methylnaphthalene	67.10	1298	tr.	roasted cereal,hay like.
2-vinylnaphthalene	71.82		0.1	sweet, fragrant.
Diethyl phthalate	85.78	1565	0.1	
Tetradecanoic acid	94.97		1.4	slightly oily,waxy.
Octadecane	97.14	1800	0.6	musty/mouldy,cereal like.
Phenanthrene	98.33		tr.	
Di(2-methylpropyl) phthalate	102.14		0.9	
Nonadecane-1-ene	102.30		2.2	
Nonadecane	103.22	1900	1.3	fragrant
Hexadecanoic acid	108.12		7.2	
Eicos-1-ene	108.66		0.3	
Dibutyl phthalate	109.09	1922	8.2	
Eicosane	110.99	2000	2.5	
An aliphatic acid	113.39		2.0	
Heneicosane	121.15	2100	1.1	
Octadeca-9, dienoic acid (i.e. linoleic acid)	127.84	16.4		
Docos-1-ene	134.63	1.1		
Docosane	138.49	2200	10.9	musty/mouldy,leathery.
Tricosane	147.45		0.6	
Tricosane	153.21	2300	1.8	sweet
Tetracosane	178.37	2400	1.2	
Pentacos-1-ene	212.87		0.9	
Pentacosane	227.05	2500	0.8	
Dioctyl phthalate	234.66		0.5	

* Adopted from "Volatile components of chayote" by G.MacLeod, 1990.

1.2.2. *Plant growth retardants* - Their regulatory actions on growth, metabolism and yield :

Manipulation of growth and development of plants for agricultural and horticultural practices is an absorbing interest to the plant physiologists. Prehistoric people were able to check the unwanted excessive vegetative growth of some crop plants by simple method of grafting, detopping or by imposing artificial starvation treatment. It is with the gradual scientfic and technical developments, scientists are able to understand the internal history of life through a window called hormonal mechanism. We are now quite aware of the fact that growth, development and yield of the crop plants are regulated by different plant growth regulators.

Cathey (1964) in his excellent review termed "growth retarding chemicals" or "growth retardants" to a new class of organic chemicals which can retard or defer plant growth and development. He defined that the growth retardants are the chemicals which can suppress the overall growth and metabolism of plants by slowing down cell division and cell elongation without altering their gross morphology.

Growth retardants can substitute manual prunning, grafting or any mechanical means for controlling vegetative growth. Agriculturists and horticulturists of the present day recommend these chemicals to utilize as efficient tools for modifying plant type to some desirable direction without any formative effects. The growth retardants have broad spectrum of utilization. At higher concentrations the retardants can check unwanted and excessive vegetative growth in respect of length, size, mass etc. and thus save labour (Nightingle, 1970), make plants lodging resistant (Weaver, 1972; Rogers-Lewis and Jarvis, 1976), low temperature resistant (Irving, 1969; Marth, 1965), less susceptible to disease development (Sinha, 1964; EL-Fouly, 1966). They have significant importance for having deferral property of senescence of monocarpic crops causing desirable growth, metabolism and yield of plants (Gill *et al.*, 1974; Guardia, 1974).

Late forty is the first introductory period of the growth retardants. There are four important groups of retardants so far discovered. They are : i) nicotiniums, ii) quaternary ammonium carbamates, iii) phosphonium and iv) hydrazines. Among these four, nicotinium compounds were first discovered in 1949 by Mitchell *et al* where the most active

compound was 2,4-dichlorobenzyl nicotinium chloride (2,4-DNC) and later on Wirwille and Mitchell (1949) reported another type of chemical having retarding property and called it as "quaternary ammonium carbamates". AMO 1618 is the most active compounds from this quaternary ammonium carbanates group of retardants. Existance of phosphonium compounds was reported in 1955 (Anonymous) and a year later Preston and Link (1958) established the retarding property of several phosphonium compounds. Phosphon (chemically known as 2,4-dichlorobenzyl tributyl phosphonium chloride) is the most active phosphonium retardant. In 1960, Tolbert established a new group of growth retardants designated as quaternary ammonium compounds. Chlorocholine chloride, abbreviated to CCC, falls under this group. Riddle *et al.* (1962) reported that substituted maleamic and succinamic acid in foliar application retarded the growth of legumes, vine crops, potato and ornamental plants. The compound most frequently in use is the N-dimethylamino succinamic acid, designated differently as Alar or B_{995} or B_9 or SADH. The hydrazines constitute the fourth group of retardant and BOH was the first of several compounds of this group capable for inducing pine apple to flower (Gowing and Leeper, 1955). In 1975, Bocion *et al.* introduced a new chemical which in many ways mimics morphactins, a potent growth suppressor. It is physiologically active as a growth regulator. It is monosaccharide in nature bearing trade name Atrinal, common name dikegulac-sodium, chemical name, 2,3:4, 6-di-O-isopropylidene-alpha-L-xylo-2-hexalofuranosate. It is produced as an intermediate product in the commercial synthesis of L-ascorbic acid. Various physiological responses induced by this chemical on a wide range of plants caused to establish it as a growth hormone of retardant class. The pinching property of this chemical evokes great attention of horticulturists and agriculturists in various parts of the globe (Arzee *et al.* 1977; Bhattacharjee *et al.*, 1986).

Mechanism of action of growth retardants is still not conclusively established. The property of retardants lies on its different types of action on different plant species or in the same species at different cellular levels. It is found that the retardants usually exert influence through the interference of GA-biosynthesis(Baldev, *et al.* 1965; Westing 1965). Hence, these are also called "antigibberellins" by many authors as they can suppress GA-induced growth and also reduce endogenous GA-level. However, Tolbert (1960) opined that the retardants are GA antagonists rather than antigibberellins because no structural similarity exists between retardants and gibberellins, and thus no competitive

inhibition is possible. But the works of Halevy and Wittwer (1965); Halevy and Shilo (1970) and Sebanek and Hink (1966) established that some retardants could enhance the endogeneous GA-level and cause promotion of growth. Again, retardants are able to reduce the growth of Avena coleoptile sections (Kurashi and Muir, 1963), sunflower hypocotyl sections (Knypl, 1964; 1966), wheat seedling growth (Norris, 1966) and *Avena* leaf sections (Cleland, 1965). They cause retardation of leaf senescence (Richmond and Lang, 1957; Osborne, 1967). According to Lockhart (1962) retardants should be considered as antimetabolites rather than antigibberelins and antiauxins. Recently, Grossmann (1990) stated that plant growth and differentiation are regulated by phytohormones that presumably exert their influence on particular metabolic reactions in the target tissue via receptor molecules. Dicks (1980) pointed out two types of phenomena during the application of growth retardants on appropriate concentration, such as, i) inhibition of shoot growth (plant height, internode elongation, leaf area) with unchanged number of internodes and leaves along with intensified green leaf pigmentation and ii) maintenance or slight promotion of root growth (Kuchenbuck, *et al.* 1988). Apart from morphological effects of growth retardants, there are numerous reports on physiological alterations along with yield improvement in various crops (Grossmann, 1990).

Recent studies of Rademacher (1990) established the idea of economic importance of the growth retardants. The widespread use of the retardants by agriculturists and horticulturists seemed prospective for yield improvement. Application of the retardants on crop plants could induce some desired characters which include : i) improving lodging resistance and canopy structure of plants (Gill, *et al.* 1974; Hasan, *et al.* 1975). ii) reducing excessive vegetative growth of plants and favouring reproduction efficiancy, iii) controlling the growth of trees, bushes, hedges and amenity grasses, (iv) improving the quality of the seedlings for mechanical transplantation, (v) retention of seed vigour and viability for longer duration (Bhattacharjee, 1984; Bhattacharjee *et al.*, 1986). During the last four decades or more numerous contributions have been accumulated with the growth retarding chemicals. Now-a-days, many researchers from the different parts of the world are concentrating their field of work on higher productivity of the agricultural and horticultural crops using growth retardants. There are a lot of reports on extensive works done with conventional retardants like CCC, SADH, AM0 1618, 2,4-DNC, Phosfon-D, etc. but work with dikegulac is rather scanty in the literature. Bocion,Sachs,

Purohit, Bhattacherjee and some others contributed variously working on this less explored retardant.

1.2.2.1. : Seedling growth and metabolism :

It was reported that the combined treatment of GA_3 and MH enhanced the seedling growth with stimulation of catalase, peroxidase, polyphenol oxidase activities (Sangeeta and Varshney, 1991). They further added that the higher concentrations of MH considerably decreased catalase activity, stimulating IAA-oxidase thereby resulting in poor germination and seedling growth. Sircar (1987) observed that MH treatment inhibited growth in *Oryza sativa*. Bhattacharjee (1984) also showed that MH treatment exhibited the same inhibitory effect on *Helianthus annuus*. Ram *et al.* (1977) observed that morphactin (100 ppm) inhibited growth of seedlings of *Phaseolus radiatus* L. Increase in peroxidase activity by morphactin treatment was accompanied by inhibition in the quantity of reducing sugar, sulphydryl, chlorophylls, and carotenoids and inhibition in α-amylase activity (Ram *et al.* 1977). Morphactin reduced the hypocotyl and radicle length as well as the dry weight of whole seedlings of *Trigonella foenum-graecum* (Balasimha *et al.*, 1978). Significant inhibition of plant height, reduction of leaf number and stem circumference were observed at 5000 µg/ml CCC treatment in sunflower seedlings (Bhattacherjee *et al.* 1984). Dorrel (1973) reported that CCC application during germination of sunflower had no effect on final height but application at lag phase of growth caused reduction in height at all concentration used but application at log phase of growth caused reduction in height at high concentrations only. Pretreatment of seeds with CCC prior to forced aging treatment slowed down the fall of germination and field emergence as well as checked the loss of DNA and RNA levels and that of activities of dehydrogenase and catalase enzymes during storage deterioration (Rai *et al.* 1992; 1995; Chhetri, *et al.* 1993). Enhanced production of insoluble and soluble carbohydrate over initial level was also noted during the earlier phases of sunflower seedlings growth (Bhattacharjee 1984). Reduction in growth of cauliflower seedling by CCC application was observed by Knypl *et al.* (1979). Balboa (1980) conformed the supporting observation of CCC-induced inhibitory effect on root and hypocotyl growth of seedling of *Lactuca sativa*. GA_3 overcame the inhibition induced by CCC or AMO-1618 only in the hypocotyl. While, Kamp and Nightingle (1979) found that SADH and CCC-induced inhibition was

insignificant in *Zinnia elegans*, and the germination of seeds was not also affected by the two retardants. CCC arrested chlorophyll synthesis in cotyledons of many plants like *Hirschfeldia incana* (Negbi and Rushkin, 1966), cucumber, pumpkin (Knypl, 1969), lettuce (Knypl and Chylinska, 1972), barley (Berry and Smith 1970) etc. Knypl (1969) in his observation cited that CCC, B-nine, Phosfon-D reduced the content of chlorophyll by 50%. However, CCC, Phosfon -D and Coumarin, in spite of their small effect on the growth of pumpkin cotyledons, inhibited accumulation of chlorophyll by about two-thirds of the control value. And GA_3 and BA could successfully reverse the inhibitory effects of the retardants brought about in cucumber, but neither GA_3 or BA effectively reduced the inhibition of chlorophyll synthesis in pumpkin. He noted that the synthesis of protein was inhibited up to 50% by CCC, SADH and Phosfon-D and also to a lower extent by AMO-1618. Potassium salts completely reversed the inhibitory effects of CCC on the synthesis of chlorophyll and protein. Khan and Faust (1966) found that some growth retardants enhanced protein synthesis as found in young barley seedlings.

Na-dikegulac is reported to induce retardation of seedling growth of a number of plants. Arzee *et. al.* (1977) showed that overall seedling growth of zinnia, sunflower and chrysanthemum was affected with regard to the shortening of internode, abnormal growth of leaves and disruption of apical dominance. Axillary shoots were developed as a result of 750 µg/ml dikegulac application in zinnia and intervascular chlorosis of leaves was apparent. But, later on, the leaves got revived and regreening occurred. In their investigation they also showed, by whole plant autoradiography, that dikegulac rapidly moved towards acropetal direction and triggered its physiological action from the shoot tips, Shemy (1978) reported that 0.3µg dikegulac per seed treatment of *Citrus* elongated the seedling growth by 20% more than control seedlings. He stated that the concentration more than 10^{-3} M dikegulac expressed distinct inhibitory effect. However, he found some gibberellin like activity at low concentration of dikegulac in rice, lettuce and cucumber seedlings. In sunflower (Purohit 1979; 1980a; 1980b). *Avena sativa* (Purohit and Chandra, 1980), *Brassica compestris* (Purohit, 1980c), sunflower, safflower, soybean, gram (Rai, *et al.* 1995) dikegulac showed inhibitory effect on seed and seedling growth and metabolism at different concentrations. Purohit (1980a, 1980b), observed that in *Helianthus annuus and Brassica compestris,* the inhibitory effects were more pronounced in respect of radicle and hypocotyl length and the reduction was found

concentration dependent. The radicle turned brown and became curved, stunted and swollen (Purohit, and Chandra 1981). Such inhibitory action of dikegulac on seedling growth might be presumed to be a consequence of its effect on protein and nucleic acid metabolism as well as on the level of various endogenous hormones known to regulate several facets of growth and development (Porohit, 1981). Purohit and Chandra (1980) found that dikegulac causes negative geotropic responses in many crop plants like *Helianthus annuus, Brassica campestris, Glycine max, Zea mays and Allium cepa*. Exogenous supply of IAA to dikegulac-treated roots could reverse the negative geotropic response (Purohit, 1980). He further reported that dikegulac adversely affected chlorophyll biosynthesis, reduced protein and sugar contents, inhibited the growth of primary as well as lateral roots. GA_3 treatment could effectively overcome inhibition of growth and chlorophyll synthesis in *Avena*.

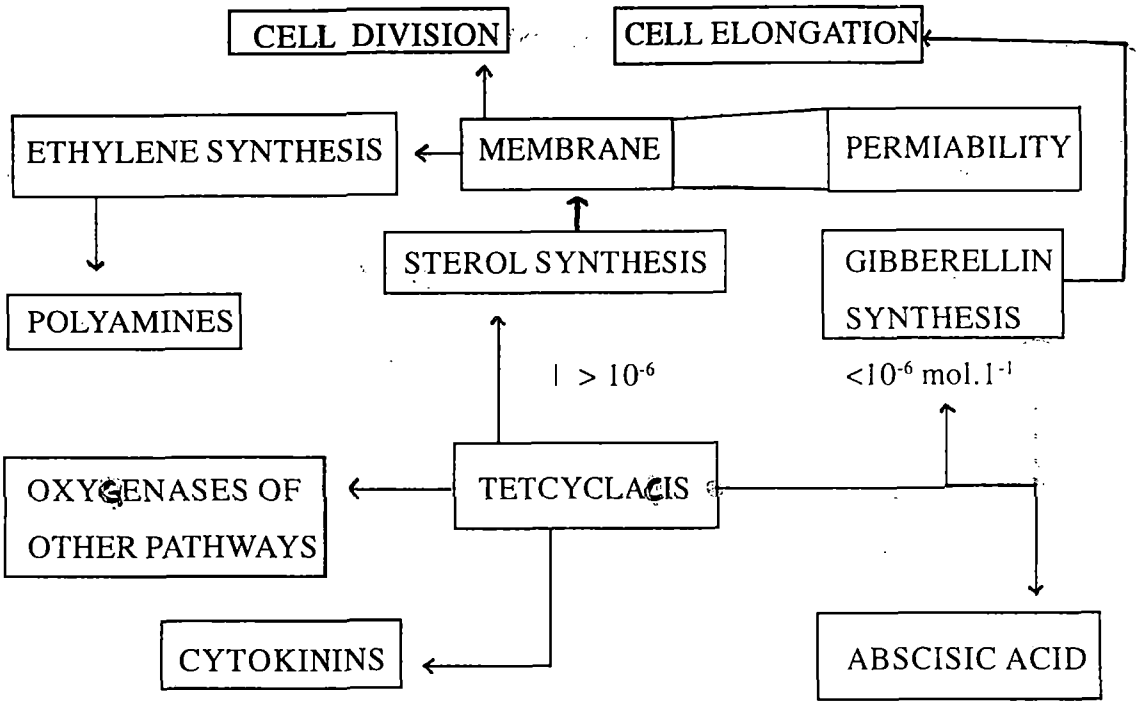
Findings of Bhattacharjee and his associates (1985; 1986; 1989; 1992; 1993; 1995; 1998; 1999) established wide spectrum of dikegulac uses. Bhattacharjee and Gupta (1981a) reported that dikegulac effect on retardation of plant growth was transient as the inhibitory effects erased sooner or later. But persistance of the chemical effect was quite apparent when it was foliarly applied at the log phase of plant growth. Bhattacharjee and Gupta (1984) reported that dikegulac showed differential respones on modifications of some growth and metabolism in dwarf sunflower cultivar. The pretreatment of *Oryza sativa*. L cultivar Ratna seeds with the chemical reduced the leaching of soluble carbohydrates and the loss of RNA during accelerated aging. Further, the chemical pretreatment to gram, soyabean, sunflower, safflower (Rai, *et al.* 1995), french bean, pea, lentil, and millet (Chettri, *et al*, 1993) seeds, significantly arrested the leakage of soluble substances and checked the declining of the levels of some vital cellular components such as carbohydrate, protein, RNA, chlorophyll content, and total dehydrogenase enzyme and also of the percentage of TTC-stained seeds. However, there is still much to be learned about this least explored chemical on various other physiological roles and their mode of action on various facets of plant growth and metabolism.

1.2.2.2. : Plant growth and metabolism

There are well established documents on the role of growth retarding chemicals on the modification of plant growth and development (Cathey, 1964; Krishnamoorthy, 1981).

The dicot species are more sensitive towards growth retardants than monocots and their pronounced effect is on the suppression of the stem elongation. Different methods of application are practiable on different plant types to ensure the effectiveness of growth retardants for desired growth modification. Application by injection method is the well experienced process (Sterrett, 1979) and seems more suitable for some plants. However, the mode of treatment, time of treatment specific concentrations and also treatment at a particular growth stage of plant species by different retardants exhibit different results and plays vital role on modification of grwoth, metabolism and yield of plants (Bhattacharjee, 1984).

The shortening of shoots under the influence of growth retardants are directly related to the activity of meristematic areas of the stems. Dicks (1980) however, found that the activity of the apical meristem, which leads to the formation of new internodes and leaves, was slightly changed. Sachs and his associates (1960;1964;1975) were able to demonstrate on stems of chrysanthemum that after treatment with growth retardant, the number of mitotic figures decreased and cell division activity in subapical meristems was diminished. Graebe (1987) and Rademacher (1990) observed the most striking results of growth retardant application in plants. Firstly, the compounds reduce the content of biologically active gibberellins particularly in young growing plant. Secondly, the growth retardant induced action can be substantially compensated by addition of gibberellin. Thirdly, in cell free systems of gibberellic acids biosynthesis the oxidative steps from ent-kaurene to ent-kaurenoic acid are specially blocked. These reactions are catalysed by a cytochrome P-450 containing monoxygenase, the kaurene oxygenase. Finally growth retardants can directly influence the cell elogation and cell division processes in shoot growth depending on the concentration of the chemical. Grossmann (1990) stated that the main action of the growth retardants on plant species is to inhibit the gibberellin biosynthesis. He proposed a model on inhibition of gibberellin biosynthesis.



(Adopted from the mini review of Klaus Grossmann on Plant Growth Retardants as tools in Physiological Research, 1990).

Cathey and Stuart (1974) reported that the foliar spray or as a soil drench application of CCC reduced the internode length of chrysanthemum for eleven weeks. Samen (1970) described that soil drench application of CCC was more effective than foliar application.

Observation of Lovett and Orchard (1974;1976) pointed out that when sunflower plants were treated with CCC at the two leaf stage showed a significant reduction in height three weeks after spraying but the effect dissappeared at maturity. CCC was shown to be a potent inhibitor of GA synthesis (Ninneman *et al.*, 1964; Lockhert, 1962). GA and CCC have opposite action on anthocyanin synthesis in raddish seedlings (Jain and Yadava, 1981). They found that anthocyanin synthesis in the raddish seedlings was enhanced by 10^{-4} - 10^{-1} M CCC while the presence of GA_3 $2.9x10^{-5}$M decreased the anthocyanin synthesis. The total free amino acid level showed a large increase in the seedlings after CCC treatment. GA_3 reduced the amino acid level. Chromatographic observation revealed that CCC treatment enhanced the level of phenylalanine. However, they also reproted that CCC treatment caused inhibition of carbohydrate level. CCC remarkably influence the growth in cereals and the effect reflects on the crop yield (Roger-

Lewis and Jarvis, 1976). Caldicott (1966) supported this by demonstrating that there were reduction of growth in straw of wheat by an average of 15% while crop yield was increased. Again, Clark and Fedak (1977) revealed that treatment of CCC at three to five leaves stages of barley, oats and wheat reduced the height most. There are, however, reports that CCC even positively influence plant growth and flowering (Halevy and Shilo, 1970). Spraying of chloromequat after 65 days of sowing of wheat plant exhibited significant shortening of the lower internode and uppermost internodes (Shcherbina, 1977). Foliar spray was shown more effective than seed treatment (Hassan, 1975). During flowering, the retardants prolonged the duration of anthesis in the summer wheat (Stamp and Geisler, 1976). Ganashan and Wittington (1975) observed that CCC reduced plant height and increased tiller number in both tall and dwarf rice varieties tested, but it delayed flowering. The combined application of CCC and ethephon remarkably reduced the straw length of winter and sumemr rye (Kuehn and Linser, 1977). Majority of the plant roots are less susceptible than shoots towards retardants. Plaut *et al*, (1964) reported that spray or soil drench application of CCC, phosfon-D, phosfon-S and B_{995} within the range of optimum concentration resulted in an increased dry weight of plant roots. Cherry plant exhibited deeper root system with CCC treatment (Kolesnikov, *et al.*, 1977). CCC-induced stimulation of potato tuber growth was observed by Choudhuri *et al.* (1976). Goleniowski *et al.* (1980) demonstrated that CCC applied to the root of potato plants grown under continuous light, seems to stimulate the synthesis of endogenous gibberellins at the beginning of stolonization without modifying the stoloniferous growth of the treated plants (Dasgupta, 1976). Tuber growth of *Dahlia pinnata* was also increased by CCC and SADH treatments both CCC and SADH significantly promoted the tuberization process and thus enhanced drymatter.(Read *et al.*, 1972). Morphactin influenced in the control of growth, metabolism and redox potential in the germinating seeds of *Phaseolus* (Ram *et al.* 1977). Higher concentrations of MH inhibited the catalase, peroxidase and polyphenoloxidase activities in *Avena* plant and it enhanced the IAA-oxidase activity (Sangeeta, 1991).

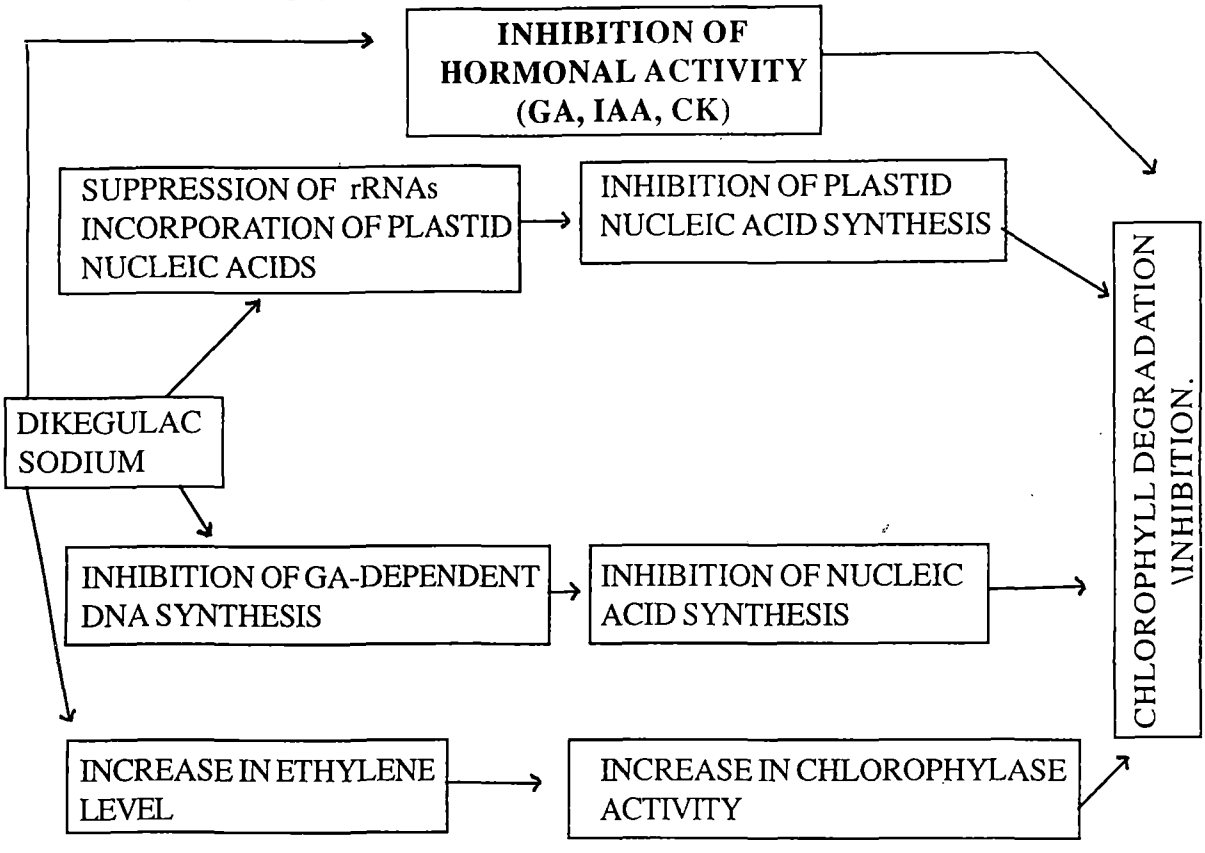
Na-dikegulac showed a significant influence on plant growth and development of different plant species (De Silva *et al.* 1976; Hield, 1978; Bhattacharjee *et al.*, 1986). Heursel (1975) and De Silva *et al.* (1976) reported a loss of apical dominance and stimulation of axillary shoot production on various azalea species after treatment of

dikegulac-Na. Purohit (1980) established that concentration of 0.05% and above of dikegulac inhibits the terminal buds. Malstrom and McMeans (1977) reported increased number of axillary shoots when dikegulac was applied to pecan trees. Inhibition of shoot elongation and axillary bud break by dikegulac application was recorded in *various plants like Thuja occidentalis, Rhododendron simsii, Berberis thunberji, Carpinus betulus, Helianthus annuus, Callistemon citrimus, Xylosma congestrum, Pyracantha coccinea, Cotoneaster pannosa and Nerium oleander.* Arzee *et al* (1977) observed that dikegulac caused distortion of leaves in *Zinnia, Helianthus and Chrysanthemum*. Purohit (1980) found that 50 to 100 ppm concentrations of dikegulac were sufficient to cause chlorotic effects in cotyledonary leaves of *Helianthus annuus* seedlings. He, further, observed that the spray of dikegulac on the seedlings caused the younger leaves to get distorted with a convoluted blade which did not expand evenly. A rosette appearance of distored leaves were observed at the tip of the shoot. All leaves continued to expand, but the lamina became yellow and developed an intervascular chlorosis. The venation, however, remained green leading to a mosaic appearance. In a few plants chlorotic and distored leaves senesced. At higher concentration (500 mg/L to 1000 mg/L) the leaves became dark brown and dry. However, as growth proceeded the chlorotic leaves regreened. Sanderson and Martin (1977) reported that in varieties of *Rhododendron* spp. dikegulac at the concentration range of 3000 to 6000 ppm inhibited shoot elongation, produced more lateral shoots than untreated plants, but the number of flowers did not increase. Treated leaves reduced in size and also malformed a little. However, three months after application, normal size as well as appearance were restored, and plant looked more compact and green. Sachs *et. al.* (1975) have shown that dikegulac inhibits elongation of internodes in a number of woody plants. Bocion and DeSilva (1976) demonstrated that aqueous solution of dikegulac was very effective as a pinching agent on azalea. They observed that one to two weeks after application of dikegulac slight chlorotic, sometimes necrotic spots appeared on the upper leaves of the shoots. The chlorosis began to disappear and the foliage regained its dark color about 8 weeks after treatment. Bhattacharjee *et. al.* (1984) reported that in 70-day-old *Helianthus annuus* plants height was significantly reduced at all the concentrations of dikegulac used (100 to 750 µg/ml). However, leaf area was decreased at the two higher concentrations and 100 µg/ml dikegulac did not influence much change in laminar area. Change in stem circumference was found inversely correlated with plant height because it was increased irrespective of

the concentrations of dikegulac used. Inhibition of DNA synthesis by dikegulac was noted in *Spirodela* and plastidial RNA was found to be more susceptible to dikegulac than cytoplasmic RNA. Dikegulac delayed the time of commencement of some important events namely, head intiation, ray floret opening, head yellowing and harvest of a dwarf sunflower cultivar (Bhattacharjee, 1984). However, such effect was apparent when application was made at the preheading stage, and seed and seedling treatment were found to be insignificant in this regard. The chemical, when applied at the preheading stage enhanced dry matter, chlorophyll and protein contents and also activity of catalase enzyme of the contributory leaves after an initial decrease noted at head development stage.

Phospon-D, B_9, CCC, AMO-1618 retarded degradation of chlorophyll in the leaf discs of *Rumex obtusifolius* (Harada, 1968). CCC and B_{995} were noted to preserve chlorophyll and protein in bean leaf as kinetin did (Kessler, 1967). CCC delayed chlorophyll, RNA and protein loss in senescing leaf discs of nasturtium; SADH at lower concentrations reduced the rate of degradation of RNA and protein but at higher concentrations the leaves tured yellow earlier than those in water control, the RNA and protein also reduced. Knypl (1967a) and Michniewiez (1968), reported that coumarin, phosfon-D and CCC were active in preserving the chlorophyll. Foilar application of 500 ppm CCC on wheat plants enhanced the RNA, chlorophyll and protein content in *Avena* leaves (Gill and Singh 1978). Deb and Mazumdar (1976) found the increment of chlorophyll and RNA in litchi plants. A reduction of chlorophyll content after CCC and ethrel application increased carbohydrate content gradually both in leaf and fruit of *Abelmoschus esculentus* (Bhatnagar, 1979). Purohit and Chandra (1980; 1981) observed inhibition of linear growth of primary leaf in *Avena sativa* when the seedlings were kept in solutions of different concentrations of dikegulac (10 to 60 mg/L). GA_3 application overcame the inhibitory effects. They also osberved, while studying dikegulac kinetin interaction, that chlorophyllase activity was increased in detached leaves of *Avena sativa* after dikegulac treatment. On the basis of all the available literature on dikegulac action on degradation/inhibition of chlorophyll biosynthesis in leaves, Purohit and Chandra (1980) proposed a model. The model suggests that dikegulac acts either by inhibiting endogenous hormonal (GA, IAA and cytokinin) activity or by interacting with hormonal biosynthesis or by supressing rRNA incorporation into plastidial nucleic acid and its synthesis or by inhibiting GA-dependent DNA biosynthesis which decreased protein

content necessary for chlorophyll biosynthesis and in addition by its direct involvement in increasing chlorophyllase synthesis.



(Proposed model for possible modes of action of Dikeulac-Sodium on chlorphyll degradation/inhibition).
(Adoped from Purohit and Chandra 1981)

Bhattacharjee (1984) observed that dry matter, chlorophyll and protein contents as well as activity of the enzyme catalase of contributory leaves of sunflower were remarkably reduced at 500 and 750 µg/ml dikegulac treatment. Effects of dikegulac and GA_3 on potato sprouting revealed that dikegulac delayed sprouting emergence and inhibits further growth of shoots while GA_3 has hastened the same (Purohit, 1980). Such delay caused by dikegulac may be due to antagonostic GA level which consequently inhibits growth activities by GA_3.

1.2.2.3. Senescence and crop yield :

Plant senescence is the deteriorative process which leads to the death of whole plant, organ, tissue or a single cell. This process is genetically controlled mechanism

supported by environmental factors. Senescence process influences different plant organs under different circumstances with variable effectiveness. Generally, it is found that old and basal leaves of a plant may be senescent while the young and more apical structures are actively growing. It is now well documented that senescence is directly associated to decrease of chlorophyll and protein or increase of their degration (Beevers, 1976; Osborne, 1967; Shaw *et al.*, 1965; Woolhouse, 1978). Apart from the cytokinin stimulation in inhibition of senescence mechanism, plant growth retardants play a significant role in inhibition process of both isolated and intact leaves of many plants (Dey and Jana, 1988). Weaver (1972) observed that plant growth retardants performed more efficiently than cytokinin in delaying senescence as observed by Mothes (1959). Biswas *et al.* (1990) observed that ABA accelerated senescence of foliar and reproductive organs, while kinitin delayd senescence of leaf only in *Ophioglossum vulgatum*. Tezuka *et al.* (1980) reported that spraying of CCC on "kyoho" grapes maintained higher photosynthetic activity in leaves and both chlorophyll -a and -b contents were remarkably increased which indicated that CCC strongly potentiated the grape plants by retaining the vital physiological processes. Niimi (1974) speculated that CCC might regulate the activity of ribulose-1, 5-bisphosphate carboxylase. CCC treated plants produced higher content of chlorophyll per unit leaf area deferring senescence in leaves producing higher amount of cytokinin (Skene, 1968; 1969). Coombe (1967) and Purohit (1981) found darker green leaves in *Vitis vinifera* and *Helianthus annus*, after CCC-treatment. Shedding of flowers and berry drops in grape plants could be inhibited by CCC treatment (Coombe, 1967; Tezuka, 1981; Naito, 1972) which enhanced berry set percentage and decreasing of berry size. CCC caused a substantial alleviation of localised senescence i.e. abscission. In "kalyan-sona-227" variety of wheat CCC maintained higher levels of chlorophyll, nucleic acid and protein as well as potential of whole plants and these reflected in the well being of the yield contributing components (Gill, 1978).

Bocion *et al.* (1975) established that dikegulac treatment induced petiole abscission in number of plant species. The chemical plays a prominent role in arrestation of senescence as evidenced by higher levels of dry matter, chlorophyll and protein contents and the activity of catalase enzymes of contributory leaves which are the main assimilate transporter leaves. Dalal and Jana (1988) observed that the different concentrations of dikegulac on *Canna indica and Coccinia cordifolia* under detached condition increased Hill reaction

activity, chlorophyll and protein contents, dry matter percentage in biomass and decreased tissue permiability over control. At the concentration of 1000 µg/ml in *Canna* and 500 µg/ml in *Coccinia* pronounced maximum inhibition of senescence, while above 1500 µg/ml stimulated senescence indicating that dikegulac at low concentrations is a potent inhibitor of leaf senescence.

So far the experimental plant, *Sechium edule*, is concerned, no reports are available on different physiological parameters. However, there are few reports on the senescence on *Sechium edule* by antioxidant and phytohoromones. Dey and Jana (1988) observed that application of antioxidants like ABA, Ferrous sulphate and mercaptoethanol significantly retarded the senescence of leaves *Sechium edule* in detached condition under dark condition. The increment in the levels of chlorophyll and protein and the activity of DCPIP-Hill reaction and dry weight was noticeable with antioxidant treatment. Further, Lama (1988) observed that the treatment with phytohormones on the detached leaves of the plant *Sechium edule* during senescence caused significant increase of soluble, insoluble and total carbohydrate contents, while RNA, dry weight and the activities of catalase and alkaline pyrophosphatase were decreased. The induction of senescence was checked temporarily in presence of kinetin, IAA and GA_3 whereas ethrel enhanced senescence.

There are variable reports on retardant-induced changes of crop yield. Lovett and Kirby (1971) observed that CCC treated plants exhibited no effect on yield. However, Hassan *et al.* (1975) demonstrated that CCC in all doses caused retardation of plant height and increased grain yield per plot (10-15%) in a wheat cultivar. Zeidan and El-Fouly (1976) observed that both early and late CCC treatments increased grain yield and number of spikes/M^2 of Egyptian and Mexican wheat. Spraying with 500 to 1000 ppm CCC increased the number of umbels and seeds upto 23% of *Pimpinella anisum*. El-Fouly (1977) reproted that treatment of CCC, B-995 and AMO-1618 on cotton plants increased the balls per plant and the effect was found to be most pronounced at B-995. Appelby *et al.* (1966) reported that seed treatment with CCC on a wheat variety showed positive response on crop yield. Higher crude protein, reducing, non-reducing and total sugar contents found in *Avena* grains with CCC treatment suggested the influence of the chemcial on seed yield and grain weight. As dikegulac is a potent height shortening agent of plants (Bocion *et al.*, 1975) the efficacy of this chemcial on alleviation of

undersired height was tested by Bhattacharajee (1984) on a tall and a dwarf sunflower cultivar during moonsoon period when maximum incidence of lodging occurs. He showed that dikegulac regardless of its concentrations used reduced the severity of head lodging in both the cultivars. Dikegulac was most successful on the tall variety with respect to arrestation of lodging and consequently of improvement of the yield attributes like percent filled seeds and average yield per plant. However, such effect of dikegulac on the lodging behaviour and yield attributes of sunflower was noted only when application of this chemical was made at the log phase of the sunflower i.e., at the preheading stage, and no such effect was apparent when dikegulac application was made at seed and seedling stage. Bhattacharjee and his associates stated that the prolongation of seed filling period in conjuction with arrestation of leaf senescence in sunflower cultivar cumulatively caused yield improvement (Bhattacharjee, 1984; Bhattacharjee *et al.*, 1984, Bhattacharjee *et al.*, 1986). They also reported that some growth retardants including Na-dikegulac could efficiently defer seed senescence also under storage and thus maintain storage potential of a number of agriculturally important seed species (Bhattacharjee and Gupta, 1985; Bhattacharjee and Choudhuri, 1986; Bhattacharjee and Bhattacharyya, 1989; Chhetri *et al.*, 1993; Rai *et al.*, 1995 Bhattacharjee *et al.*, 1999).

Information collected from the literature cited above, it is concluded that the plant growth retardants efficiently induce various modifications on growth, metabolism and yield of different plant species. Depending upon the concentrations of growth retarding chemicals and their applications at various growth stages of plants the responses often become defferential. However, chemical manipulation of plant growth and development using plant growth regulators is an improtant field of research which needs to be thoroughly explored keeping in mind the productivity of crops. In many crop plants growth retardants efficiently obviate or alleviate negative yield attributing factors. From biochemical point of view retardants are also found to be desirable chemical manipulants for maintaining higher metabolic status and plant potential for a longer period which consequently result in enhancement of crop yield. Hence, during the present days strategy is being made by agriculturists to obtain higher productivity by utilizing growth retarding chemicals in many plants in addition to conventional field practices as well as amending crop plants with chemical and organic fertilizers.

Now, it is a challenge to the modern reserachers to devise better strategies for efficient utilization of this chemical group keeping in mind the cost-effective aspect of productivity.