



*Summary*

## SUMMARY

Different germplasms of chayote (*Sechium edule* Sw., Cucurbitaceae) were collected and ten varietal types (named alphabetically A to J) were recorded after a thorough exploration of different chayote growing altitudinal regions Darjeeling hills. Unlike other cucurbits the above ground leafy plant is annual and underground tuberous part of the plant is perennial and all parts e.g. tuberous roots, fruits and tender shoots are used as human food. Phenological studies showed that the leafy plant survives around 160 days and within its life span it shows some distinct developmental phases which include : field emergence phase, first leaf emergence phase, seedling phase, sapling phase, flower initiation phase, fruit formation phase, senescence phase and death phase. Each phase is clearly distinguished by a few key features. Plantation of different varietal types of chayote starts from February. Vigorous vegetative growth and fruiting takes place during the monsoon months of June and July and harvesting of fruits is completed by October each year.

A plant growth retardant Atrinal or Na-dikegulac (2,3 : 4-6-di-O-isopropylidene- $\alpha$ -L-xylo-2 hexalofuranosate), was applied at three different stages of chayote plant viz., at sprouting stage of fruits (intact or defleshed fruit treatment), at sapling stage (30-day-old plants) and at preflowering stage (60-day-old plants). The chemical-induced changes on growth, metabolism and yield were analysed at different developmental stages of the plant. Atrinal-treated plants at the preflowering stage were given a supplementary treatment with GA<sub>3</sub> (100  $\mu$ g/ml) or kinetin (100  $\mu$ g/ml) at the flowering stage of plant development (70-day-old). Effect of such combined treatments on vegetative as well as reproductive growth, metabolism and crop yield were analysed at fruiting and senile stages of plant development.

**Intact or defleshed fruit treatment at sprouting stage :** Treatment of intact or defleshed sprouting fruits with Atrinal (500, 1000 and 2000  $\mu$ g/ml), resulted in significant increase of chlorophyll and protein contents in leaves at the seedling and sapling stages only, and subsequent changes recorded at preflowering, fruiting and senile stages were statistically insignificant. Almost an identical trend of changes were noted when soluble carbohydrate, insoluble carbohydrate, RNA and DNA levels in leaves were analysed at the different developmental stages. Corresponding changes in the activities of catalase and peroxidase

enzymes in leaves were recorded. The growth retardant, irrespective of its concentrations, enhanced the activities of these two enzymes at the two initial observation periods i.e., at seedling and sapling stages, and thereafter the chemical effect was nullified. Conversely, activities of IAA-oxidase and RNase enzymes were suppressed by Atrinal treatment at the seedling stage only, and this inhibitory action did not persist at subsequent analyses done at preflowering, fruiting and senile stages.

Atrinal-induced biochemical changes in leaves were associated with the changes of growth parameters like vine length and stem circumference. Regardless of concentrations Atrinal retarded vine length and increased stem circumference, and the effects were found significant up to sapling stage only.

Yield attributes recorded in terms of fruit number, fruit weight and tuberous root weight as well as days to onset of plant senescence remained unchanged by the retardant treatment given at the sprouting stage of fruits.

Atrinal treatment was found to be equally effective or ineffective in case of plants raised from intact or defleshed fruits as in both the cases the chemical-induced effects were significant at seedling and sapling phases of plant development. At subsequent analyses Atrinal-induced changes were found to be ineffective in both kinds of plant samples raised either from intact fruits or defleshed fruits.

Thus, in case of fruit (intact or defleshed) treatment the retardant could render only a transient effect on the growth and metabolism of chayote plants and such changes were not at all reflected in yield attributes.

**Foliar treatment at sapling stage :** Foliar application with the same concentrations of the growth retardant at the sapling stage (30-day-old plants) caused a significant reduction of chlorophyll, protein, soluble and insoluble carbohydrates as well as DNA and RNA levels in leaves at the initial observation period of sapling stage on 40-day-old plants. The inhibitory

effects were found transient and the chemical subsequently augmented the levels of these cellular components.

Activities of the enzymes catalase and peroxidase were declined shortly after foliar application of the Atrinal but this retardation effect was not only alleviated quickly but the activities were found even higher at Atrinal-treated plant samples analysed at fruiting and senile stages. A reverse change in the activities of IAA-oxidase and RNase was recorded where the retardant-induced transient increase in the enzyme activities were followed by a consistent decrease till senile stage. Unlike the retardant-induced differential biochemical changes with respect to growth stages of the plant, vine length was retarded and stem circumference was increased by Atrinal treatment and such retardation or enhancement effects were found to be maintained throughout the observation periods.

Atrinal showed a tendency towards deferring leaf senescence of chayote plants but among all the concentrations Atrinal 1000 and 2000  $\mu\text{g/ml}$  showed<sup>a</sup> significant senescence deferral effect. Again a significant increase of yield components like fruit weight and tuberous root weight per plant was recorded in Atrinal treated plants.

Thus, unlike fruit (intact or defleshed) treatment, effect of foliar treatment with the growth retardant was found to fairly persist till senile stage and the changes of the growth and biochemical parameters were associated with a substantial increase of yield components.

**Foliar treatment with Atrinal at preflowering stage and Atrinal followed by hormonal treatment of flowering stage :** Atrinal, irrespective of its concentrations, resulted in increase of chlorophyll, protein, soluble carbohydrate, insoluble carbohydrate, RNA and DNA levels in leaves of chayote plants at both fruiting and senile stages. However, the retardant-induced increases were further augmented by  $\text{GA}_3$  and kinetin treatments at flowering stage.

Like the changes of the above biochemical parameters, almost identical trend of changes was recorded in catalase and peroxidase activities, and GA<sub>3</sub>- as well as kinetin-induced additive effect was found to be very prominent. Changes of IAA-oxidase and RNase activities were found to be reverse to that of peroxidase and catalase. Here the retardant-induced decrease of IAA-oxidase and RNase activities were reduced to a further extent in supplementary treatments with GA<sub>3</sub> and kinetin.

Atrinal-induced reduction of vine length was overcome to some extent by the second treatment with GA<sub>3</sub> and kinetin. On the other hand, the retardant increased stem circumference and the effect was found additive in supplementary treatments with kinetin. Atrinal showed a tendency towards deferring of leaf senescence and senescence deferral effect was found significant in the single treatment with Atrinal (1000 and 2000 µg/ml) and in combined treatments with Atrinal and kinetin. Yield attributes like fruit weight and tuberous root weight were augmented by the retardant treatment and this augmentation was much more remarkable in combined treatments particularly with kinetin. Atrinal, however, failed to increase particularly the number of female flowers per plant while combined treatments with GA<sub>3</sub> and kinetin remarkably increased both female and male flowers. Again, the combined treatment with Atrinal and GA<sub>3</sub> exerted the best response on increasing female flowers than such treatment with Atrinal and kinetin..

Regulatory action of the growth retarding chemical Atrinal and its promising role on augmentation of crop yield are discussed.



*Review of the  
Literature*

## REVIEW OF THE LITERATURE

Review work of this investigation is dealt under two major captions: 1. On the biology and prospects of chayote (*Sechium edule* Sw.)-the experimental plant and, 2. On Atrinal (Sodium dikegulac) – the key plant growth regulant.

### 1. ON THE BIOLOGY AND PROSPECTS OF CHAYOTE (*SECHIUM EDULE* SW.) THE EXPERIMENTAL PLANT

#### Botanical Characters

The chayote suggests the cucumber rather than any other of the cultivated plants of the same family, but is a larger and more vigorous plant, climbing widely by means of numerous branched tendrils. The leaves are strongly three-angled or lobed, with the broadly cordate base also showing two or four sharp corners. The leaves as they stand in nature are deeply concave, with the apex sharply decurved. The surface is rather rough, but there are scarcely any hairs, and the color is a deep, fresh green. The whitish veins are rather conspicuous.

The pistillate flowers are solitary, but otherwise not greatly different in general appearance from the much more numerous staminate blossoms. The latter are borne on special branches, which are often described as “short whorled, long-stalked, axillary racemes,” though it is not clear that they are either racemes or whorled, the actual structure consisting merely of single small clusters at the nodes of a shortened and leafless, branch. Both filaments and styles are connate into a central column, of which the anthers appear as lobes, while the stigmas are more closely set together to form a small head. The ratio of male and female flowers per flowering leaf axial becomes roughly 25:1. Pollination occurs by bees or insects, pollen grains are polycotylate, 8-9 colp <sup>7</sup>ate, oblate, 52-55  $\mu\text{m}$ , spiniferous; chromosome complement is  $n=14$ .

The ovary is always one-celled, with a single ovule. It is mealy-pubescent when young, becoming spiny with maturity in some varieties. The mature fruits are more or less compressed, as though built over the large flat seed. They are also, in general, pear shaped, in that they are narrower near the point of attachment and broader toward the apex. In addition to the spines, which, however, are not always present, the surface of the fruit is usually more or less uneven, and has, in addition, several deep longitudinal grooves or channels, more pronounced toward the ends, and in some varieties nearly obliterated near the middle. In different varietal types of chayote found in Darjeeling hills the shape, size, colour, texture varies.

### **Vitality of the Fruit**

The fruit of the chayote presents unique physiological and morphological adaptations. It is comparable, perhaps, with that of the mangrove (*Rhizophora*), though the similarity extends only to the fact that germination may take place before the seed falls from the parent plant. The fruit of the mangrove is adapted for taking root in the soft mud, into which it penetrates by means of the long, pointed radicle, but in the chayote, which must fall upon drier ground, a projecting radicle would be broken off. Instead of putting forth a radicle, the apex of the mature seed is extruded from the fruit only far enough to expose the tip of the hypocotyl, from which arises a tuft of small roots. The plumule escapes laterally from between the cotyledons, which are not further drawn out or separated from the fruit, and the latter, instead of drying up or decaying at maturity, or before the germination of the seed. Unlike other cucurbitaceous members the fruits of chayote are hardy and can withstand adverse environmental situations. By virtue of having such unique property they can be safely stored for a prolonged period without losing the quality and food value (Lama, 2000).

That such an adaptation should arise in the Cucurbitaceae is even more strange than it would have appeared in many other families, owing to the well-known perishability, or at least limited vitality, of the fruits of this group. The chayote

constitutes, as it were, the antithesis of the balsam-apple (*Momordica*), the flesh of which opens and begins to disintegrate, almost by deliquescence, as soon as the apical seeds have matured, and while those at the base of the fruit are still far from ripe.

### **The Seed and Germination**

The chayote further deviates from the normal type of the Cucurbitaceae in its one-seeded character, and in the fact that the seed coats are obsolete or very imperfectly differentiated. At maturity the seed is embedded in the middle of the fruit and entirely enclosed. But before germination the seed grows so that the apex of the cotyledons is pushed further toward the base of the fruit, while the 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 surface, representing the original area of the seed, which remains closely in contact with the fleshy and undifferentiated seed coats. It is in this area of the cotyledons, presumably, that the absorption of the nutritive material from the fruit into the seedling takes place (Cook, 1901).

In the chayote a whole fruit functioning as endosperm during an extended period in which it is capable of general vegetative activity. Possibly, however, the nutritive aspect of its utility may be equaled or even exceeded by its importance as a means of storing moisture to assist in carrying the young plant through periods of drought. The large tuberous roots which meet this requirement for the more mature plant are said not to be formed until the second season. A third possible advantage of the species is that by being able to send out without delay a vine several feet long, seedlings of the chayote might be able to make connection with the soil without the seed or fruit having come in contact with the ground at all. Such an adaptation would be of obvious utility in permitting a large-fruited species to maintain an existence where the ground is covered with tangled masses of vegetation (Cook, 1901, Mukhia *et al*, 1982).

The keeping qualities of fleshy fruits and vegetables are dependent upon the vitality of the protoplasm of the cells. Many fruits can be kept for considerable periods under favourable conditions and the time can be artificially extended by cold storage. In nature, the chayote seems to furnish the instance of a fruit which normally continues alive even after the germination of the contained seed and after separation from the parent plant. The readiness with which the seed of the chayote germinates is probably the only obstacle to its exploitation as a commercial product. What determines the germination is not exactly known, and it may be found that if kept sufficiently dry and cool condition there will be no difficulty from this source. In Mexico, according to Dr. Edward Palmer, it is considered an easy matter to preserve the fruits indefinitely by packing them in dry sand. It is customary to allow the seeds to germinate before planting the fruits being placed for this purpose on the shelves of living rooms or in other sheltered places. About New Orleans a similar practice is followed, fruits kept for planting being wrapped in paper and laid away in cool dark cellars or storerooms during the winter. Chayotes shipped from Algeria to the markets of Paris and London are said to bear shipment well, even when eight or ten days on the journey, and to remain for a long time in good condition (Cook, 1901). In Darjeeling hills the local cultivators, after harvest, keep fruits in dry air for 15-30 days and then store in dry sawdust or millet husk in dark and cool place (Shil, 1990, Lama, 2000).

### **Varieties of Chayote**

Cook (1901) and others reported the existence of two very distinct varieties of *Sechium edule*, the first with green colored fruit and the other with white one. There exists differential flower colors of the varieties i.e., the white variety has green colored flowers and large in size than the green variety with white and small flowers. Cook (1901) also reported 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 of the two varieties grown in Madeira. the cream colored or white variety was larger in size. Engels (1983) reported

eleven varieties of chayote from Central America. Lama et al (1994) reported ten varieties of chayote from Darjeeling hills.

### **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, 0.2% 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% starch in young apical shoots. Analysis of the chayote fruit flesh showed 3.3% soluble sugar and 0.2% starch, in the seed 4.2% soluble sugar and 1.9% starch was found on wet weight basis. They observed that fruit contains 15 and 25 time more soluble sugar than tuberous root and apical shoots respectively whereas starch 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 starch. 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 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 starch content was higher in the seeds ( $85 \mu\text{g} \cdot \text{mg}^{-1}$  dry wt.) than the flesh ( $75 \mu\text{g} \cdot \text{mg}^{-1}$  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  $\alpha$ -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 different enzymes like ATPase, F-1,6 dipase, G-6-Pase and G-1-Pase were observed in chayote. ATPase 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,  $\alpha$ -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 existence 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 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.

It has been demonstrated that endosperm and cotyledons synthesized gibberellins simultaneously 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<sub>15</sub>, the 3-propyl-or 3-acetyl-GA<sub>4</sub>, the 3-propyl or 3-acetyl-GA<sub>15</sub>, the 3-propyl-or 3-acetyl-GA<sub>4</sub>, the 3-propyl or 3-acetyl-GA<sub>7</sub>. The function of these conjugates are not well understood. The *S. edule* GA glucoside is biologically active in the lettuce and rice bioassay. 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 ovule 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 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 composition of chayote, MacLeod (1990) reported different, volatile components of chayote. A total of 61 components were identified and four were partially characterized and these are represented in the following table.

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

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

## **Distribution**

The chayote is a popular and an important home grown food in tropical and subtropical regions of the New World. It is well spread and cultivated in West Indies, throughout Southern Europe, Southern United States and Russia, East Indies, Australia. In Asian countries, it is cultivated in Sri Lanka, Mascarene Islands, Malayasia, Nepal, Philippines, India and so on. In India it is mostly cultivated as vegetable in the hills of India. In the state of Megalaya, Sikkim, Uttar Pradesh, Himachal Pradesh, West Bengal, Karnataka and Maharastra of India this crop is grown luxuriantly in temperate and subtropical regions (Chakravorty 1973). The crop can be grown with relative ease due to its adaptability to a wide range of climatic conditions. Louis and others reported the existence of the plant in areas at sea level to regions with altitudes between 4000 to 7500 ft. The crop can tolerate cold but succumbs to prolonged hard frost and hence it can be grown even in high altitudes.

## **Common and scientific names of chayote**

Unlike other plants, this plant has many common names. Although it is not very widely distributed, it has a lot of localized names. Cook (1901) assumed that variable names of the plant was due to slow and gradual introduction in new communities of the world. And the name has forgotten before the fruit had obtained standing in the markets and thus required a popular designation. In India, the differential naming of the plant is due to varied regional languages. A list of common names of chayote is given herewith (Cook, 1901).

**CAHIOTA** – Recorded in Lowe's "Flora of Madeira".

**CAMOCHAYOTE** – A Mexican name for the edible root of the chayote.

**CHAHIOTA** – Another Madeira name.

**CHALLOTE** – Recorded by Seeman from the Isthmus of Panama. In a subsequent mention the more correct form, chayote is used.

**CHAYOTA** – A West Indian form of the following, used by Jacquin and others.

**CHAYOTE** – The modern Mexican and now generally preferable name of which several others are more corruptions.

CHAYOTE FRANCES – According to Jacquin, this name was applied in Cuba to a small, smooth variety of the chayote, but Maza associates it with the Sponse gourd (Luffa).

CHAYOTE PELON – A mexican name, evidently for a smooth variety. “ pelon” meaning bold.

CHAYOTESTELE – A mexican name, for the edible root of the chayote.

CHAYOTITO – A mexican name for a variety of chayote (Herrera).

CHAYOTITO GACHUPIN – A mexican variety of chayote, “gachupin means “fine” or “elegant”.

CHAYUTLI – The ancient Aztec name as recorded by Hernandez in the sixteenth century. This is said to signify a “ head bristling with spines” or a “ squash covered with thorns”.

CHINCHAYOTE – A mexican name for the edible root of the chayote.

CHIOTIE – Used in Belt’s Naturalist in Nicaragua.

CHOCHO – The prevalent name in the British West Indies and in Australia.

CHOKO – A Queensland variation of the preceding.

CHOU – CHOU – Recorded by Mr. Faichild as in use among the creoles of Louisiana.

CHOUCROUTE – From the French colonies particularly Reunion; evidently a compromise between the preceding and ‘chayote’.

CHOW – CHOW – An English rendering chou-chou.

CHRISTOPHINE – Reported from the French West Indies and France.

CHUCHU – The Brazilian name; evidently a further corruption of ‘chocho’.

MIRLITON – In use among the creole population of Louisiana.

ONE – SEEDED CUCUMBER – Apparently inverted by the English-speaking residents of New Orleans.

PERINELLAN – Madeira (Lowe).

TALLOTE – Known only from Porto Rico.

TAYOTE – The Porto Rico modification of the name chayote.

UPOPO – A Mexican variety of chayote (Herrera).

VEGETABLES PEAR – British West Indies (Grisebach).

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Apart from the above, there are several common names for chayote in different parts of India, which are listed hereunder :

**ESKUSH** - Indian name for the varieties of chayote which is the corruption of English word for 'squash'. This name is common in Darjeeling hills.

**QUASH** - A Bengali name for chayote and it is also the corruption of English word 'squash'.

**SOH-KWASH** - Name used by Khasi - hill people for chayote of eastern India.

**SEEMA - KATTIRIKKAI** - South Indian name, designated by Tamilnadu people.

**SEEMA - BADAIVE** - Name for chayote, in the Karnataka state.

### **Scientific Name :**

In 1760, Jacquin proposed the first botanical name '*Sicyos edulis*' to the chayote under the binomial system of nomenclature. Later in 1780, the same author changed the above name and called it by the latinized form of the native name, *Chayota edulis*. After 20 years Swartz used the name, proposed by Patrick Browne in 1756, *Sechium*. This name was not employed under the binomial system until the time of Swartz. There are three opinions as to nomenclature of the plant; the first one emphasises the binomial name proposed by Swartz, that is, *Sechium edule*; the second one gives importance on Jacquin's name as the oldest acceptable binomial designation, while third opinion would favour *Sechium* simply because older as a generic name than *Chayota* or *Sicyos* without regard to the binomial system. In 1763 Adanson had also proposed to replace Brown's name *Sechium*, with the vernacular chocho, but this name was not popularised.

### **Economic importance and Commercial Prospects**

Chayote has immense economic potential and commercial prospect as a vegetable crop in many countries of the world including India. This is mainly because of its easy cultivation procedure, considerable food value of all its plant parts like fruits, tuberous roots and leafy shoots, prolonged storage capacity of its fruits and tuberous roots. It is often called a zero management crop with high yield potential.

The chayote is one of the most important of the indigenous economic plants of Mexico, and that it is the superior of the potato in the quality and quantity of its products. Its culture is rapidly increasing in Mexico and it is one of the most important of cultivated plants since it furnishes palatable and wholesome foods which can be produced with extreme cheapness. The single seeded character of the fruit is a serious impediment to its rapid introduction and multiplication in culture (Cook, 1901). In tropical America, France, Cuba, Australia, North Africa the cultivation of this vegetable crop is being popularized gradually.

In Algeria, although chayote was introduced as early as 1845, and highly recommended in 1860 by M. Hardy, the then director of the experimental garden of Algeria, it has increased in popularity very slowly for many years, and now it has become a commercial product of serious importance. Bulk export of chayote takes place from Algeria to different countries. Its utility as a substitute for the base of the true artichoke in high-priced dishes is a suggestion that it will find other places in the elaborate cuisine of Paris and other large cities of Europe.

### **Endogenous hormonal levels of chayote**

Maturing seeds of *Sechium edule* Sw. contain very high levels of free and conjugated gibberellins throughout seed development; both free and conjugated gibberellic acids (GAs) are abundantly present also at the completion of seed development. Various studies regarding the identity and the endogenous levels of seed GAs have been published. In 1983, Ceccarelli and Lorenzi first reported on the structure and concentration of biologically active GAs in the endosperm and cotyledons of seeds at different developmental stages. A detailed analysis of the GAs present in the different seed components of *Sechium edule* Sw. was also reported by Albone *et al.* (1984). Biosynthesis of major endogenous GAs was also shown in cell-free systems prepared from both endosperm and cotyledons of maturing seeds (Ceccarelli and Lorenzi, 1983). The purification and identification of the conjugated GAs present in the seed were reported (Lorenzi and Ceccarelli, 1986). These conjugates were biologically active in the

standard GA bioassays (lettuce hypocotyl and dwarf rice). Consequential to the studies Ceccarelli and Lorenzi (1992) investigated the hormonal relationship between fruits, developing seeds and the mother plant and between the mature seed and the derived plantlet. The authors showed that removal of fruit and seed teguments was ineffective on plantlet growth while the removal of cotyledons arrested growth under short days. They suggested that the control of early plantlet growth by cotyledons under short day conditions may be mediated by GAs.

Immature seeds of *Sechium edule* Sw. are a rich source of GAs, the structure and approximate concentration of the different GAs present in seed tissues have been determined (Lorenzi and Ceccarelli, 1983; Albone, et al., 1984; Lorenzi and Ceccarelli, 1986). Previous work (Ceccarelli and Lorenzi, 1983) has demonstrated that cell-free systems prepared from endosperm and cotyledons of immature seeds are able to convert MVA to the endogenous C<sub>19</sub>-GAs present in the tissues.

Seeds of *Sechium edule*, a viviparous species, contain high levels of gibberellins and cytokinins throughout seed growth and development (Lorenzi and Ceccarelli, 1983; Lorenzi, et al., 1988). Endogenous levels of ABA in the same seeds have been also investigated (Gnagnarini and Lorenzi, 1985), and absence of hormone accumulation in coincidence with the maximal seed growth as observed in other species (King, 1982; Ackerson, 1984), has been noticed. Paolo, et al, (1989) reported on the quantification of ABA in integuments and nucellus of pollinated and unpollinated *Sechium* ovules. They showed that in unpollinated ovules ABA concentration was higher in comparison to that in pollinated ovules of *Sechium*.

## 2. On Atrinal (sodium dikegulac) – the key plant growth regulant

In agricultural and horticultural practices manipulation of growth and development is an absorbing area of research by the plant physiologists. People of even prehistoric times learned to check excess vegetative growth of crop plants simply by detopping or by incapacitating them to uptake adequate nutrients and water from soil i.e. by rendering them to experience some sort of artificial starvation. In recent years a new class of organic chemicals has appeared with the special characteristics that they can retard or defer growth processes in plants, and these are termed 'growth retarding chemicals' or 'growth retardant' (Cathey, 1964). Dikegulac-sodium (sodium 2,3: 4,6-di-O-isopropylidene- $\alpha$ -L-xylo-2-hexalofuranosate) or ATRINAL (trade name), has established itself as a potent chemical belonging to this group. Since its first report by Bocion *et al.*, (1975) from Dr. R. Maag Ltd., Switzerland, a considerable volume of work on different aspects of growth, development, metabolism and yield of a number of plant species has been accumulated (de Silva *et al.*, 1976; R zee *et al.*, 1977; Malstrom and McMeans, 1977; Sanderson and Martin, 1977; Hield *et al.*, 1978; Zilkah and Gressel, 1980; Wilson and Nell, 1983; Purohit and Chandra, 1983; Jaafar, 1984; Bhattacharjee *et al.* 1986). Particularly due to its chemical pinching property, the chemical evokes attention of quite a good number of horticulturists in different countries of the world. It is now being used as a very good substitute of manual pinching by arborists, highway maintenance divisions and landscape contractors for woody and herbaceous perennials.

Dikegulac, produced as an intermediate product in the commercial synthesis of L-ascorbic acid, is a sugar hormone which is monosaccharide in nature and of the different salts, sodium-linking to it was found to be most effective with respect to exhibiting the hormonal activity. Hence sodium-dikegulac (or dikegulac-sodium) was recommended as a potent synthetic growth hormone. On the basis of physiological responses elicited by dikegulac, it seems that the chemical is close to morphactins.

Among other growth retardants, it has also some resemblance with cycocel (CCC) and Alar (SADH) with respect to their common biological action although structurally the chemical is quite different from them (Bhattacharjee *et al* 1986). Arzee *et al.* (1977) demonstrated that it works counter to auxins or to gibberellins but it is neither an anti<sup>au</sup> auxin or an antigibberellin in true sense. The chemical possesses extremely low bee, fish and mammalian toxicity; it is not irritant to eyes and skin (de Silva *et al.*, 1976). Physically it is white, odourless, solid with a m.p. 300°C and does not exhibit photosensitivity. Na-dikegulac is stable in aqueous solutions at pH 7 and above, highly soluble in water, methanol and ethanol and less soluble in chloroform, acetone, cyclohexane and hexane.

A thorough literature search revealed that since its first report in 1975 over 200 publications have appeared on dikegulac covering various research fields. The present review is based on the reports of some of the experimental works available with this chemical during the last decade or more investigation.

### **Seed germination and hypocotyl growth**

A few reports are available on the effect of dikegulac on the germination behaviour of seeds. Dikegulac does not significantly alter germination percentage of sunflower (Purohit, 1979; 1980a; 1980b) or *Brassica campestris* (Purohit 1980c) seeds but it specially inhibits the radicle and hypocotyl elongation at the low concentration range (50 to 250 µg/ml) of the chemical tested. Using a higher concentration range of 500 to 1000 µg/ml, Bhattacharjee and Gupta (1985) reported that dikegulac slowed down the process of seed germination of sunflower but did not affect the final germination percentage i.e. the inhibitory effect was merely transient. However, hypocotyl length was markedly reduced irrespective of the concentrations used. Results obtained with different crop plants (*Helianthus annuus*, *Brassica campestris*, *Glycine max*, *Zea mays* and *Allium cepa*) revealed that the reduction in hypocotyl and radicle lengths was concentration

dependent. The radicle turned brown and became curved, stunted and swollen (Purohit 1979; 1980b,c; Purohit and Chandra, 1981). There are some reports that loss of germinability of jute (Bhattacharjee *et al.*, 1986), sunflower (Bhattacharjee and Gupta, 1985) and rice (Bhattacharjee and Bhattacharyya, 1989) seeds under conditions of accelerated ageing treatment was found higher in dikegulac-pretreated seeds. Hypocotyl length of 6-day-old sunflower seedlings, raised from seeds pretreated with dikegulac was found to be reduced to 29.1%, 16.8% and 11.6% over control seedlings at 2000, 1000 and 500  $\mu\text{g/ml}$  respectively (Bhattacharjee, 1984).

Along with seed germination, associated biochemical changes in cotyledons of germinating sunflower seeds were found to occur during the process of germination (Bhattacharjee, 1984; Bhattacharjee and Gupta, 1985). He observed low level of soluble carbohydrate in cotyledons of dikegulac-pretreated seeds up to 96 h after seed-soaking, while an inverse picture was noted as to the changes of insoluble carbohydrate level. Free amino acid content as well as DNA and RNA levels were found to be low up to 72h of seed germination, while in later observation periods a clear increasing trend was apparent. Dikegulac distinctly reduced  $\alpha$ -amylase activity at the initial observation period but this inhibitory effect was erased subsequently. All such observations can explain the temporary inhibitory action of dikegulac on germinability of sunflower seeds. The author suggested that owing to subdued availability of soluble carbohydrate, amino acids, nucleic acids as well as  $\alpha$ -amylase, transient hindrance of seed germination was apparent in sunflower.

### **Seedling growth and metabolism**

There are reports in the literature that dikegulac influences seedling growth and metabolism of a good number of plant species (Bocion and de Silva, 1976; Arzee *et al.*, 1977; Shemy, 1978; Purohit, 1979, 1980a,b and c). Arzee *et al.*, (1977) showed that the overall seedling growth of zinnia, sunflower and chrysanthemum was affected with

regard to the shortening of internodes, abnormal growth of leaves and disruption of apical dominance. The authors showed that in zinnia axillary shoots were also developed as a result of 750 µg/ml dikegulac application and intervascular chlorosis of leaves was apparent. However, in all the three species dikegulac-induced convoluted and chlorotic leaves later regreened. Histoautoradiographic studies showed that DNA synthesis was inhibited in the apical meristem, and normal cytohistologic zonation was no longer apparent. In their investigation, they also showed by whole plant autoradiography that dikegulac moved towards acropetal direction and triggered its physiological action from the shoot tips. The authors suggested from their data that dikegulac acted selectively on meristematic cells in the apex and developing leaf primordia, and a minute amount of the chemical was sufficient to effect changes in the apical development. Shemy (1978) reported that *Citrus* seedlings, which were treated with up to 0.3 µg dikegulac per seed, elongated their shoot 20% more than the control seedlings. He found some gibberellin-like activity of dikegulac at its low concentration on rice, lettuce and cucumber seedlings. The author, however, stated that dikegulac had distinct inhibitory effect at concentrations higher than  $10^{-3}$  M. Several reports revealed that dikegulac reduced the seedling growth of sunflower (Purohit, 1979, 1980a,b), *Avena sativa* (Purohit and Chandra, 1980), *Brassica campestris* (Purohit, 1980c), or *Glycine max* (Purohit and Chandra, 1980) and the effect was found to be concentration dependent. Purohit (1979, 1980a,b) reported that concomitant with the reduction of seedling growth, dikegulac adversely affected chlorophyll biosynthesis, reduced protein and sugar contents, inhibited the growth of primary as well as lateral roots. The authors also showed that dikegulac-induced inhibition of growth and chlorophyll biosynthesis in *Avena* could be effectively overcome by  $GA_3$  treatment. They also noted that 50 to 100 µg/ml dikegulac was sufficient enough to cause chlorotic effects in cotyledonary leaves of *Helianthus annuus* seedlings.

Bhattacharjee (1984) studied the developmental behaviour as well as metabolism of seedlings, raised from dikegulac-pretreated seeds. He showed that dikegulac-inhibited

height, leaf area and dry weight of 14-day-old sunflower seedlings. A proportional shift in seedling metabolism was also noted after seed pretreatment with dikegulac. Results showed that chlorophyll and protein levels as well as catalase activity in leaves of seedlings were found to be low, whereas activities of IAA-oxidase and protease enzymes were high in dikegulac-treated samples. He also showed from this experiment that when observation was made on 28-day-old plants all such deleterious effects on growth and metabolism were erased.

There are reports that growth retardants temporarily exert an inhibitory effect on growth and metabolism (Cheema *et al.*, 1975; Ben Gad *et al.*, 1979). It is presumed that owing to dikegulac-induced impairment of nucleic acid, chlorophyll and protein biosynthesis, seedling growth was adversely affected. However, after an initial set back, the seedlings restored their normal functional life by antagonizing the initial deleterious plant processes, as was evident from complete revival of growth and metabolism of sunflower at later observation periods.

### **Stress Tolerance/Avoidance**

Some sporadic reports are available on the regulation of stress tolerance/avoidance capacity of dikegulac. Bhattacharjee and Choudhuri (1986) using two cultivars of jute (*Corchorus capsularis* L. cv. JRC 212, and *C. olitorus* L. cv. JRO 632) showed that dikegulac-treated seedlings gained some potential for withstanding unfavourable environmental stress. It was shown from this experiment that the health of seedlings, which experienced water-stress treatment after having a prior pretreatment with dikegulac, was superior to seedlings which underwent same stress treatment but without any seed treatment. Results showed that after 7 days of water-stress treatment by immersing the root system of jute seedlings in PEG-6000 (Polyethyleneglycol) solution for 48 h, both height and dry matter content were higher in seedlings, which were raised from dikegulac-pretreated seeds. They also noted that chlorophyll and protein contents in

leaves of the untreated and dikegulac-pretreated seedlings increased after 7 days of water-stress treatment but the increases were much more remarkable in seedlings developed from dikegulac-pretreated seeds. Such changes were associated with higher activities of catalase and superoxide dismutase enzymes in leaves of seedlings raised from dikegulac-pretreated seeds. Higher activity of these scavenger enzymes (Fridovich, 1976) are indicative of higher plant potential rendering plants tolerant against environmental stresses. Hence, these are regarded as reliable indices for evaluation of stress tolerance capacity of a plant. Elstner (1982) reported that free radicals participate, chiefly in the form of activated  $O_2$  species such as superoxide ( $O_2^-$ ) or  $H_2O_2$ , in several electron transfer reactions of normal cell metabolism and are usually controlled by the appropriate protective mechanisms, such as superoxide dismutase, catalase and peroxidases. There are also reports that catalase and superoxide dismutase activities of detached wheat and rye leaves decline with concomitant decrease of chlorophyll and protein during ageing (Kar and Feierabend, 1984). In fact, adaptive responses of plants towards environmental stresses are indicative of their high vigour and these are reflected in metabolism through gene expression (Hochachka and Somero, 1973). Thus from the available information on the growth and metabolic behaviour of seedlings, raised from dikegulac-pretreated seeds, it seems quite apparent that dikegulac strengthened the defence mechanism by stimulating the activities of the free-radical scavengers which consequently resulted in substantial alleviation of the damaging effects of environmental stress. Biswas and Choudhuri (1986) reported that pretreatment of *Vigna* seedlings with dikegulac through root systems significantly improved water status, maintained membrane integrity, chlorophyll and protein levels of water stressed seedlings. Water stress-induced proline accumulation in *Vigna* was also significantly inhibited by this treatment. From their observation they came to the conclusion that dikegulac acts as a potential hardening agent against water stress.

## Dikegulac as a Chemical Pinching Agent

Perhaps the most important and well-established property of dikegulac, so far known, is its effect on pinching and stimulating branch initiation of a good number of plant species. The chemical quickly reduces or disrupts apical dominance of plants being mobilized to the shoot apex within a short period after treatment, and thus strongly retards plant growth (Bocion *et al.*, 1975; Arzee *et al.*, 1977). Its effect on the production of profuse axillary branches of many ornamental plants is amply documented (Bocion and de Silva, 1976; Sanderson and Martin, 1977; Malstorm and McMeans, 1977; Hield *et al.*, 1978; Larson, 1978; Agnew and Campbell, 1983; Arnold and Aldrich, 1983).

Sachs *et al.* (1975) demonstrated inhibition of shoot elongation and axillary bud-break on many shrubs and trees like *Xylosma congestum*, *Pyracantha coccinea*, *Callistemon citrinus*, *Cotoneaster pannosa* and *Nerium oleander*. Green-house trial with *Eucalyptus globulus*, *Fraxinus uhdei* and *Ulmus parviflora* indicated that dikegulac is a useful inhibitor for landscape trees. Bocion *et al.* (1977, 1978) found that dikegulac is effective as a growth retardant and pinching agent for *Gerbera jamesonii*, *Cyclamen persicum*, *Fuchsia*, *Pachystachis lutea* and *Begonia elatior*. These plants were of uniform and compact shape after dikegulac treatment. Sanderson and Martin (1977) reported that in 4 varieties of *Rhododendron, sps.*, dikegulac at the concentration range of 3000 to 6000 µg/ml inhibited shoot elongation, produced more side shoots than untreated plants, but the number of flowers did not increase. However, in *Gerbera jamesonii* production of profuse axillary branches were associated with formation of increased number of flower. Treated leaves reduced in size and also malformed a little. Three months after application, normal size as well as appearance were restored and plants looked more compact and green. DeSilva *et al.*, (1976) found that foliar application with 0.4 to 0.6% dikegulac was effective as a pinching agent under commercial growing conditions on all the azalea cultivars tested. They noted that within 4 weeks after treatment, the axillary shoots began to elongate, stem diameter was also increased in all the cultivars except 'R Ambrosius'. Information of Orson and Kofranek (1978) revealed that foliar spray of dikegulac effectively pinched most of the varieties of

*Rhododendron* tested which include : 'California Sunset', 'Chimes', 'Dogwood', 'Golris', 'Kaute Erwin' and 'Rose Queen', and the pinching was very effective under certain environmental conditions. The resultant shoots greatly diminished at the highest concentration of the chemical. Although dikegulac was found to be phytotoxic to immature leaves near the growing point, this did not detract from the quality of the plants. Malstrom and McMeans (1977) showed that foliar application of dikegulac after bud break stopped shoot growth and caused chlorosis of many leaflets in pecan trees, but application before leaf-fall delayed bud growth and promoted lateral shoot development.

Heursel (1975), Bocion *et al.*, (1975) and Bocion and de Silva (1976) demonstrated that an aqueous solution of dikegulac is very effective as a pinching agent on azalea (*Rhododendron simsii*). They observed that one to two weeks after application of dikegulac, slight chlorotic some times necrotic spots appeared on the upper leaves of the shoots. Axillary shoot elongation was initiated after four weeks of dikegulac application, and chlorosis began to disappear and foliage regained its dark colour about eight weeks after treatment. A better pinching effect was obtained with higher spray volumens but it depends upon the growth stage of azalea (Bocion *et al.*, 1975). Wise and Fonteno (1980) working with *Petunia hybrida* reported that when seedlings were given foliar application with 400 µg/ml Atrinal, greatest number of branches were found within acceptable foliar toxicity limits. Greater and lesser concentrations produced fewer branches and higher concentrations also produced more toxic effects. In *Capsicum annum* both hand pinching and chemical pinching with dikegulac increased branching in all the three cultivars tested (Mattia, 1983; 1984). Arnold and Aldrich (1983) reported that when peach plants were applied by handgun with 500, 1000, 1500 and 2000 µg/ml dikegulac, tree height and width decreased but lateral branching increased with increasing concentrations of dikegulac. Barua and Gupta (personal communication) have used dikegulac on safflower at sapling stage and found that the chemical of the concentration of 500 µg/ml significantly increased the number of secondary branches along with reduction in plant height. In Wing bean dikegulac altered the plant habit and ~~dwarfing~~ <sup>dwarfing</sup> effect was very significant (Das Gupta *et al.*, 1985). From all such reports, it has been well established that dikegulac is a suitable chemical pinching and shoot-inducing

agent. Hence, it is recently being effectively used as a substitute of hand pruning and thus save manual labour.

### **Plant Growth and Metabolism**

**Growth :** Effect of dikegulac on the alteration of plant growth has been studied by a number of workers (Bocion *et al.*, 1975; deSilva *et al.*, 1976; Hield *et al.*, 1978; Orson and Kofranek, 1978; Bhattacharjee, 1984; Bhattacharjee and Gupta 1984a,b; Mattia 1984). Bocion *et al.*, (1975) reported that dikegulac retarded the growth of a wide range of plant species which included cereals, cultivated as well as weed grasses and woody plants. Morphological observations revealed that dikegulac produced some toxic effects on the shoot apex and on the young growing leaves of sunflower, zinnia and chrysanthemum which was evidenced from the convoluted nature, yellowing and narrowing of leaf lamina along with the appearance of some necrotic spots (Arzee *et al.*, 1977). Similar observations on morphological abnormalities of some other plant were noted by Purohit (1980c), Kawabata and Criley (1982), and Bhattacharjee *et al.*, (1984).

Using a tall and a dwarf sunflower cultivar Bhattacharjee (1984) reported that when foliar application was made just before head initiation stage of sunflower plant height was reduced in both the tall and dwarf varieties at the higher concentration of 500 and 750  $\mu\text{g/ml}$ . Dikegulac at 100  $\mu\text{g/ml}$  reduced the height of tall cultivar only, and the magnitude of inhibition was low in dwarf cultivar. He also showed that such effect of dikegulac is more pronounced over two other height shortening agents CCC and SADH. Production of leaves was equally affected in both the cultivars at the two higher concentrations. Results also showed that stem circumference was significantly increased at 100 and 500  $\mu\text{g/ml}$  regardless of cultivars concerned, while 750  $\mu\text{g/ml}$  was found to be ineffective in this regard. Leaf area was decreased at all the concentrations in case of tall cultivar, but interestingly in dwarf cultivar this was increased at 100  $\mu\text{g/ml}$  dikegulac. When application of dikegulac was made at the seed or seedling stage of the the two cultivars, retardation of growth was found merely transient, because when observations were made beyond head initiation stage of sunflower retardation effect was completely

erased, and dikegulac-treated plants looked as good as control ones. So, attempt to check excess plant growth by dikegulac application at seed and seedling stages became futile. From such observations, it seems quite apparent that growth retardation of sunflower becomes effective, in terms of persistence of retardation effect, only when application was made before head initiating stage of sunflower. It might be mentioned in this context that the unbalanced plant type of this photoneutral, oil-yielding crop renders the plants susceptible to lodging particularly during rainy season. This is mainly due to (i) shallow and sparsely branched root system in comparison to the heavy foliage – and massive capitulum – bearing aerial part (ii) extremely strong apical dominance rendering plants tall (iii) weak and herbaceous stem (iv) less developed secondary tissues in stem causing subdued mechanical strength. All these deleterious plant characteristics are responsible for the reduction of crop yield, and hence this crop needs the modification of its unbalanced plant type for higher productivity. The author showed from his investigation that dikegulac can substantially alleviate some of these disadvantageous features by chemical manipulation with dikegulac. But selection of the appropriate stage of application and that of the optimum concentration of the chemical are the important criteria for obviating the handicaps in case of sunflower. In fact, log phase of plant growth in sunflower starts just before head initiation stage (Dorrel, 1973). Hence, plant growth was successfully manipulated by dikegulac application at this particular stage by disrupting its log phase. On the other hand, application of the chemical at lag phase of plant growth is of no worth, because after an initial set back when plants reach their log phase the chemical effect was completely erased and consequently plants started growing vigorously.

Shulmann and Lavee (1983) reported that growth of grapevine and olive shoots was inhibited by dikegulac-sodium. Concentrations of 500 to 6000  $\mu\text{g/ml}$  were effective in grapevine 'Perlette', while only 3000 or 6000  $\mu\text{g/ml}$  inhibited the growth of 'Manzanillo' olive shoots. They showed that the chemical reduced the size of young leaves and clusters; mature leaves, however, were not affected. Application to dormant grapevine buds caused delayed bud opening, weak growth and deformed shoots. At 3000 and 6000  $\mu\text{g/ml}$  dikegulac induced shortening of internodes and swelling of nodes of olive shoots. Adriansen and Andersen (1983) showed that *Atrinal* reduced shoot length

of *Aeschynanthus hildebrandii* about 26 cm. Making a more suitable plant height and in case of *A. speciosus* reduction of shoot length occurred up to 32 cm. In *Rieger begonia* dikegulac, chlormequat as well as hand pinching effectively reduced internode length but did not affect overall height, stem diameter, visual quality or dry weight (Agnew and Campbell, 1983). Working with 4 cultivars of greenhouse-forcing azaleas (*Rhododendron spp*) Shu and Sanderson (1979) reported that dikegulac decreased shoot length and increased shoot number but the chemical did not have a long term depressive effect on azalae shoot growth and development, because 6 weeks after treatment shoot growth increased normally. Kawabata and Criley (1982) found that dikegulac effectively suppressed the growth of *Murraya paniculata* in the spring but not in winters and the hedge did not show regrowth in summer. They further showed that spraying of dikegulac at one day after the trimming or at the budbreak mainly inhibited the first flush, and spraying at the expansion of the first leaves mainly inhibited the second flush. Jaffar (1982, 1984) from his experiment on a commercial rubber plant (*Hevea brasiliensis*) concluded that in spite of manipulation of plant growth dikegulac may be promising new growth substance for releasing dormancy of buds in budded stumps and maxi stumps.

**Metabolism :** There exists a number of reports that dikegulac, like other growth retardants, also exerts influence on plant metabolism (Bocion and deSilva, 1976; Gressel and Cohen, 1977; Zilkah and Gressel, 1978; 1979, 1980; Purohit and Chandra, 1981; Bhattacharjee and Gupta, 1981a,b and 1984a). With histoautoradiographic studies of the shoot apex Arzee et al. (1977) showed that in the apical meristematic zone of zinnia, sunflower and chrysanthemum DNA synthesis was strongly inhibited. Inhibition of DNA synthesis by dikegulac occurred on *Spirodela* and plastidial RNA was found to be more susceptible to dikegulac than cytoplasmic RNA (Gressel and Cohen, 1977).

Bhattacharjee (1984) reported differential action of dikegulac with respect to the changes of metabolic activity in directly treated and newly expanding leaves of a tall and a dwarf sunflower cultivar. Because, anabolic activity was highly impaired and catabolic activity was much more augmented in newly expanding leaves having actively dividing cells in comparison to those in the maturing leaves which received direct treatment.

There are, however, reports which indicate that dikegulac acts differentially on dividing and stationary cells as the former ones are extremely susceptible to dikegulac, while latter ones are hardly inhibited even at relatively high concentrations (Zilkah and Gressel, 1978, 1980).

In pot experiments, Bhattacharjee (1984) noted that after foliar application of dikegulac at the preheading stage chlorophyll level in the directly treated leaves was reduced over initial content irrespective of cultivar at 500 and 750  $\mu\text{g/ml}$  after 7 days of treatment. In dwarf cultivar however, reduced chlorophyll level started reviving after 14 days of treatment which was not apparent in case of the tall cultivar at least up to 21 days after treatment. Interestingly, in dwarf cultivar the low concentration of 100  $\mu\text{g/ml}$  enhanced chlorophyll content over control at later sampling periods, while the tall cultivar remained inert in this respect. As regards the changes of protein and dry matter content in the treated leaves, almost identical trends were noted. Both soluble and insoluble carbohydrate contents remained in low level over control values at 500 and 750  $\mu\text{g/ml}$  dikegulac treatment in case of tall cultivar, while in dwarf cultivar soluble carbohydrate increased at 500  $\mu\text{g/ml}$  and insoluble carbohydrate increased at 100  $\mu\text{g/ml}$ . Both DNA and RNA contents in treated leaves declined regardless of sunflower cultivars and the effect was found to be concentration-dependent. On the other hand, RNase and protease activities were greatly stimulated and catalase activity was inhibited at the two higher concentrations in the treated leaves of the two cultivars. In contrast to the dikegulac-induced biochemical changes in treated leaves, in newly expanding leaves, a drastic impairment of such metabolic variables were noted. Because, in such leaves the inhibition of anabolic activities and promotion of catabolic activities were much more pronounced than treated leaves and even the lowest concentration of 100  $\mu\text{g/ml}$  was found inhibitory. Thus, it seems quite apparent from this investigation that dikegulac exerts differential action depending upon the nature of tissues and the tall cultivar appears to be more sensitive to this chemical. This is quite in conformity with some other reported observations as well (Bocion and de Silva, 1976; Bhattacharjee and Gupta, 1981 a,b). In a separate study under field condition, Bhattacharjee and Gupta (1984a) and

Bhattacharjee et al (1984) showed that all such metabolic variable, which were affected as a result of dikegulac application, after the cessation of dikegulac application, revived completely at later periods of plant growth. This is indicative of the fact that dikegulac-induced inhibitory effects on plant metabolism does not perpetuate for longer duration.

### **Chlorophyll Degradation/Inhibition**

Inhibition of chlorophyll biosynthesis has been studied in *Zinnia*, *Chrysanthemum* and *Helianthus* (Arzee et al., 1977), *Azalea* (Bocion and de Silva, 1977a), *Helianthus annuus* (Purohit, 1979) and *Brassica campestris* (Purohit, 1980b). Purohit and Chandra (1980b) 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). Such inhibitory effects of dikegulac could be overcome when GA<sub>3</sub> was applied along with dikegulac. This reveals that GA<sub>3</sub> neutralizes the adverse effects of dikegulac by bringing about growth and chlorophyll biosynthesis to control level.

It is known that presence of GA<sub>3</sub> is essential for retention of chlorophyll in isolated leaf discs of *Rumex* (Whyte and Luckwill, 1966). At the molecular level dikegulac inhibits GA-induced DNA synthesis (Arzee et al., 1977). Studies on dikegulac-induced modulation of plastidial nucleic acid synthesis have indicated that in axenically cultured *Spirodela*, dikegulac depressed uridine incorporation into both plastidial and cytoplasmic ribosomal RNA's. GA<sub>3</sub> stimulated precursor incorporation into organellar DNA, was found to suppressed by dikegulac.

Sabater and Rodriguarz (1978), Purohit (1982a,b) studied degradation of chlorophyll during senescence of detached leaves of *Avena sativa* and *Helianthus annuus*. They indicated that chlorophyllase enzyme is responsible for such loss (Sabater, 1984). Similarly, Shimokawa (1983) observed enhancement of chlorophyllase activity after ethylene treatment in *Citrus unshiu*. Ethylene level increases after dikegulac treatment and kinetin interacts with dikegulac (Bocion and de Silva, 1977a). Purohit and Chandra (1980 d) also observed, while studying dikegulac-kinetin interaction, that chlorophyllase

activity showed an increase in detached leaves of *Avena sativa* after dikegulac treatment. They indicated that chlorophyllase is involved in the protective effects of kinetin against chlorophyll loss and correlation between chlorophyll retention and chlorophyllase level is highly significant. Therefore, dikegulac may directly enhance chlorophyllase activity or indirectly increase ethylene level which in turn would enhance the enzyme activity.

Combining together all the reports of various workers cited above, Purohit and Chandra (1980b) have proposed a model pertaining to the possible mode of actions of dikegulac on degradation/inhibition of chlorophyll biosynthesis in leaves. The model suggests that dikegulac may act either by inhibiting endogenous hormonal (GA, IAA and cytokinins) activity by interacting with hormonal-induced other growth regulatory activities related to chlorophyll biosynthesis (Arzee et al., 1977; Bocion and deSilva, 1977a) or by suppressing rRNA incorporation into plastid nucleic acid and its synthesis (Gressel <sup>and Cohen</sup> 1977) or by inhibiting GA-dependent DNA biosynthesis which decrease protein content necessary for chlorophyll biosynthesis (Arzee et al., 1977) and in addition by its direct involvement in increasing chlorophyllase synthesis and or activity induced by ethylene because the level of ethylene increases to six-fold after dikegulac-treatment (Bocion and de Silva, 1977a). Purohit and Chandra (1981) observed reversal of dikegulac-sodium induced chlorophyll degradation and chlorophyllase activity in *Helianthus annuus* by urea.

### Changes of Endogenous Hormones

A few reports are available which support that dikegulac affects the levels of some endogenous hormones like IAA, GA, ABA and ethylene in plants (Bocion and deSilva, 1976, 1977; Purohit and Chandra 1981b; Bhattacharjee, 1984). Bocion and de Silva (1976) reported that at  $10^{-3}$  M concentration dikegulac enhanced endogenous ethylene level of pea seedlings up to 6 times of the control value. Level of tryptophan, a precursor of IAA, declined gradually with the increasing concentrations of dikegulac and concomitantly IAA-oxidase activity steadily increased (Purohit and Chandra, 1981).

In pot experiments, Bhattacharjee (1984) reported that when foliar application of dikegulac was made for three consecutive days at the preheading stage of sunflower GA-

like substances was reduced to a considerable extent just after 5 days of treatment. Such a low level of GA was found to be maintained up to the observation periods of 25 days in case of 500  $\mu\text{g/ml}$  dikegulac, while at 100  $\mu\text{g/ml}$  after an initial fall, revival was recorded after 15 days of treatment. Unlike GA, ABA-like substances increased almost steadily up to 15 days of treatment and then a declining trend was noted. 500  $\mu\text{g/ml}$  was found to be more stimulatory than 100  $\mu\text{g/ml}$  in this regard. However, at the final observation, the changes of ABA level was found to be statistically insignificant. In field experiments, an interesting pattern of the hormonal (GA and ABA) changes were noted when the data were recorded at the three developmental stages of sunflower viz, head developmental stage, 50% anthesis stage and preharvest stage after foliar application of dikegulac at the preheading stage. Results showed that GA-like substances were found to be low, with respect to control value, at the head developmental stage; at 50% anthesis stage such inhibitory effect tended to be nullified and at preharvest stage the hormonal level was found to be higher in dikegulac-treated samples than in control ones. So far the changes of ABA-like substances were concerned with respect to these developmental stages the result was found to be almost inverse to that of GA.

Arzee et al (1977) assumed that the action of dikegulac may cause reduction in GA as well as GA-induced DNA synthesis. Gressel et al. (1977) speculated that dikegulac reduces auxin level or counteract the action of auxin because it reverses apical dominance, which is maintained by endogenous auxin level in plants. Like other growth promoters, dikegulac at its low concentration has been shown to produce a synergistic mode of promotion with  $\text{GA}_3$  on the callus growth of tomato (Bocion and de Silva, 1976). Shemy (1978) reported that low concentration of the chemical promoted the growth of *Citrus* seedlings, and some gibberellin-like activity was also noted by him in experiments with rice, lettuce and cucumber seedlings, Bhattacharjee (1984) noted a stimulatory action of dikegulac at 100  $\mu\text{g/ml}$  on growth and metabolism of sunflower. Hence, a proposition of dikegulac (low concentration) mediated production of growth hormones involving in synergistic action may not be ruled out. Thus, from the available information, it seems apparent that dikegulac, as its short-term effect, generally reduces the levels of growth promoters and enhances the level of growth inhibitors. But under certain instances, at low concentration it acts synergistically with some other growth

promoters and may stimulate growth possibly by increasing the endogenous hormonal level.

### **Stem Anatomy and Lignification**

Although growth retardant-induced changes of stem anatomy (Halfacre and Barden, 1968; Smolinski et al., 1972; Tezuka et al., 1980, Phelps *et al.*, 1980) and lignification (Kaplya and Moroz, 1976; Munnich and Koschuchowa, 1977; Hrebinskyi *et al.*, 1978; Khamis *et al.*, 1979) are amply documented, dikegulac-induced modification of stem anatomy and lignification is scanty in the literature. Bhattacharjee and Gupta (1984a) made such a study after foliar application of dikegulac at the preheading stage of a dwarf sunflower cultivar (*Helianthus annuus* L. cv. Modern). They showed that after 30 days of treatment dikegulac altered anatomy of sunflower stem mainly by stimulating the growth and development of secondary tissues. At the low concentration (100 µg/ml) of dikegulac cambial activity in the stem of treated plants was noted to be higher than that of untreated ones. This was evidenced from well-developed vascular tissues in each bundle as well as from the appearance of a good amount of vascular tissues both in fascicular and interfascicular regions. The activity of fascicular cambium seemed to be more pronounced than that of interfascicular ones, which resulted in disproportionate thickening of vascular tissues. But the identity of the individual bundles was more or less maintained. At the higher concentration (500 µg/ml) of dikegulac, development of mechanical tissues was found more profuse. Here, activities of both the fascicular and interfascicular cambia were more or less uniform, thereby producing a continuous thick band of secondary tissues. In contrast to low concentration, more cell layers were found in the cortical zone. Detailed observations further revealed that in treated samples the number of rows of vessels, particularly of tracheids, were increased. At 500 µg/ml, the average number of rows of tracheid and vessels were 27 and 8 respectively and at 100 µg/ml they were 12 and 6 respectively; in contrast, in untreated controls there were 8- and 4-seriate tracheids and vessels, respectively.

Bhattacharjee (1984) reported that along with the changes of stem anatomy, lignin content in sunflower stem was also higher in dikegulac-treated plants. Using three different concentrations (100, 250, 500 µg/ml) of the chemical, the author showed that in

contrast to control value lignin level was increased to 39.6%, 31.0% and 16.8% at 500, 250 and 100  $\mu\text{g/ml}$  dikegulac treatment respectively. He conclusively proved his findings from a separate experiment by measuring O.D. values of the pooled safranin of the stained, untreated and treated samples. However, as to the details of anatomical modification as well as the mechanism<sup>f</sup> controlling the process, there is much to be learned. Phelps *et al.*, (1980) reported that thickening and anatomical changes of *Salix* stem as a result of morphactin application were brought about by the synergistic effect of morphactin and endogenous hormones which greatly stimulated cambial activity. Tezuka *et al.*, (1980) noted remarkable development of xylem and phloem in the internodes of primary shoots of grapes by CCC application which, according to them, was cytokinin-mediated. Because, along with growth reduction, CCC treatment has been shown to increase the levels of substance with cytokinin activity (Skene, 1968, 1969). Infact, cytokinin-like substances are found to increase in plants as a result of reduction of apical dominance by decapitation (Sato *et al.*, 1977). Dikegulac-induced disruption of apical dominance and suppression of plant growth have been well-established. Therefore, for enhancing the cambial activity and consequent enhancement of radicle growth in sunflower, the question of involvement of dikegulac-stimulated cytokinin, might not be ruled out. Whatever might be the actual mechanism, it seems apparent that dikegulac possesses the property of anatomical modification in stem chiefly because of their effect on suppression of vertical growth of plants.

### **Translocation of Assimilates**

Hormone-directed translocation of assimilates is a well-established phenomenon (Davies and Wareing, 1965; Mulligan and Patrick, 1979; Patrick, 1979). It is generally accepted that actively growing meristems and developing organs are the potential sinks for photosynthetically produced assimilates, and that promotion or repression of apical sink may result in corresponding changes in growth patterns (Moorby, 1977; Wareing and Patrick, 1975). Growth retardants generally act through suppressing the apical sink by reducing the hormonal levels therein and consequently by hindering the acropetal mobilization of assimilates (Monselise and Luckwill, 1974; Hoad and Monselise, 1976;

Ben-Gad *et al.*, 1979). However, reports, on dikegulac-induced mobilization of assimilates are rare in the literature.

Bhattacharjee (1984) made a critical study on the translocation pattern of radioactive phosphorus ( $^{32}\text{P}$ ) after foliar application of dikegulac on sunflower and this is indicative of the chemical-induced assimilate translocation in plants. He recorded the data after feeding  $^{32}\text{P}$  to the head (capitulum) and also to the topmost leaves, but promoted it to the basal leaves and roots, the effect being concentration dependent. It is this clear that dikegulac reduces acropetal mobilization but induces basipetal mobilization of assimilates. However, acropetal mobilization was greatly stimulated by IAA, Kinetin and  $\text{GA}_3$  (100  $\mu\text{g/ml}$  each) application to the head at 50% anthesis stage following dikegulac (100  $\mu\text{g/ml}$ ) application at the preheading stage of sunflower. This is evidenced from greater accumulation of the radioactive phosphorus both in the central and peripheral regions of sunflower capitulum.

Lovett and Orchard (1976) reported from their experiment with radioactive carbon that in sunflower photosynthetic rate and assimilate translocation were reduced by CCC treatment in the upper leaves. However, greater accumulation in CCC-treated plants was noted in the roots. Monselise and Luckwill (1974) demonstrated that acropetal translocation of assimilates was hindered by SADH beginning almost immediately after treatment. Thus, from the reported observations, it may seem that like other growth retardants dikegulac probably hindered acropetal mobilization by weakening the apical sink of sunflower, and this allowed the root sinks to become capable of accepting the surplus assimilates. That such an inhibitory effect of dikegulac is hormone-mediated, can be proved from high mobilization of phosphorus in sunflower heads when IAA, Kinetin and  $\text{GA}_3$  were applied on the head at 50% anthesis stage. This is indicative of the fact that repression of apical sink by dikegulac was overcome by hormonal application of apical sink by dikegulac mobilization force resulting in greater  $^{32}\text{P}$  accumulation in the capitulum of sunflower.

## Flowering, Fruiting and Harvest Delay

Wise and Fonteno (1980) showed that at the concentration range of 200 to 1200  $\mu\text{g/l}$  dikegulac delayed flowering in *Petunia hybrida* from 1 to 10 days compared to controls. Using 3 cultivars of *Capsicum annum*, Mattia (1983) reported that both hand pinching and Atrinal (at 14.8 ml/plant) delayed the first harvest by 9 days. He also showed that Atrinal decreased yield of all cultivars at all concentrations. However, Arnold and Aldrich (1983) noted no effect of dikegulac on the changes of flowering date of peach trees, Adriansen and Anderson (1983) reported that Atrinal delays the flowering in *Aeschynanthus hildebrandii*, but it has no effect on the flowering time in *A. speciosus*.

Flowering and fruiting are markedly inhibited by dikegulac in *Alix crenata* when the plants were sprayed at the mid of the post flowering stages (deSilva *et al.*, 1976). However, the chemical stimulated flower development and flower number in *Cyclamen persicum* and *Gerbera jamesonii* (Bocion *et al.*, 1978). Foliar spray of dikegulac on *Helianthus annuus* (Purohit 1980d) before flowering hastened flowering and the effect was found to be inversely proportional to 100 and 250  $\mu\text{g/ml}$  concentrations. Beyond these concentrations delay in flowering was directly proportional to the concentrations. A very peculiar abnormality was recorded in some of the flowers developed on the plants sprayed with 250  $\mu\text{g/ml}$  dikegulac. The abnormality was decreased significantly with increasing concentrations of dikegulac. The seeds in treated plants were light brown and slightly elongated whereas in untreated plants they were dark brown in colour. Bhattacharjee (1984) noted distinct distortion of sunflower capitulum at the two higher concentrations (500 and 750  $\mu\text{g/ml}$ ) of dikegulac, which was evidenced from blunt as well as necrotic apices of the bracts of capitulum which extended inwardly and covered a large part of disc floret zone. Moreover, the thalamus never assumed the normal plate-like appearance and ray-florets failed to emerge. However, such anomalies were not apparent at 100  $\mu\text{g/ml}$  dikegulac as well as in CCC and SADH treatments. Dikegulac-induced changes in size and shape of sunflower seeds was noted and compared with two other growth retardants (CCC and SADH) after foliar application of the chemicals at the preheading stage. At 100  $\mu\text{g/ml}$  dikegulac, sunflower seed were found to be rather bold, and the size seemed to be increased a little over control. On the other hand, at 500  $\mu\text{g/ml}$

although seeds apparently look bold, they were partially filled, light and tend to appear roundish.

Bhattacharee (1984) made a study on the changes of times of occurrence of some important events in the life cycle of the dwarf sunflower cultivar after dikegulac application at seed, seedling and preheading stages of the plant. Data showed that the schedule times of ray-floret opening, head yellowing and harvest of sunflower were not deviated when treatments were made both at seed and seedling stages of sunflower. However, a little delaying effect on the incidence of head initiation only was noted. On the other hand, when dikegulac was treated at the preheading stage delaying effects on the incidence of head initiation only was noted. On the other hand, when dikegulac was treated at the preheading stage delaying effects on head initiation, rayfloret opening, head yellowing and harvest of sunflower was found to be very conspicuous, and at 500  $\mu\text{g/ml}$  of the chemical harvest was delayed up to 15 days. It was further noted that seed yield remained almost unaffected when the chemical was applied at seed and seedling stages but when treatments were made at preheading stage, yield was found to be positively or negatively influenced at 100 and 500  $\mu\text{g/ml}$  dikegulac treatment respectively.

Study on development of parthenocarpic fruit by dikegulac application was made on *Lycopersicum esculentum* (Bocion *et al.*, 1975) and *Pyrus communis* (Bocion and deSilva, 1977a). Foliar application of 300  $\mu\text{g/ml}$  dikegulac twice at 20 day intervals resulted in more fruits than single application. Bocion *et al.*, (1975) also showed that dikegulac stimulates fruit ripening. Available information, thus, reveals that in addition to the influencing effect of dikegulac on some aspects of growth, development and metabolism of plants, the chemical has some role the alteration of reproductive behaviour of plant species.

### **Senescence and Abscission**

Although there are reports that dikegulac influence the greening of leaves and rejuvenate treated plants after a transient degreening (Sanderson and Martin, 1977), reports on dikegulac-induced changes of senescence and abscission are rare in the

literature. Bocion et al. (1975) showed that dikegulac induces petiole abscission of a good number of plant species. Jana et al., (1986) studied the effects of dikegulac concentrations (100, 500, 1000, 1500 and 2000  $\mu\text{g/ml}$ ) on some senescence variables in leaves of *Canna indica* and *Coccinia cordifolia* under detached condition. They noted that treatments of 100, 500 and 1000  $\mu\text{g/ml}$  <sup>increased till</sup> activity, chlorophyll and protein contents, dry matter percentage in biomass and decreased tissue permeability over control. The inhibitory effects of dikegulac on leaf senescence were markedly pronounced with the treatments of 1000  $\mu\text{g/ml}$  in *Canna* and 500  $\mu\text{g/ml}$  in *Coccinia* during dark-induced senescence. However, at concentrations beyond 1500  $\mu\text{g/ml}$  all such senescence variables were impaired indicating that dikegulac at low concentrations is a potent inhibitor of leaf senescence, while at higher doses it promotes leaf senescence at least in case of *Canna* and *Coccinia*. Bhattacharjee et al. (1984) studied the effects of dikegulac on leaf senescence of sunflower under attached condition. They reported that dry matter, chlorophyll and protein contents as well as activity of catalase enzyme of contributory leaves (Johnson, 1972) were remarkably reduced at 500 and 750  $\mu\text{g/ml}$  dikegulac, when analysis were made at head development stage. At 50% anthesis stage, these adverse effects were overcome and at preharvest stage all such senescence variables were found remarkably high over control plants. All these are indicative of the fact that after an initial adverse effect, dikegulac-treated plants steadily rejuvenated and this was maintained for a longer duration. Thus, dikegulac may be a potential chemical for a monocarpic plant like sunflower with regard to arrestation of leaf senescence. Such senescence deferral effect of dikegulac was conclusively proved by Bhattacharjee and Gupta (1984a) in a separate study.

As regards the mechanism of dikegulac-induced deferment of leaf senescence nothing is clearly known. However, it is speculated that such effect may be cytokinin-mediated. Because, there are reports that endogenous cytokinin level increases in CCC-treated grape vines which consequently maintain higher chlorophyll level per unit leaf area (Skene, 1968, 1969). Available reports also indicate that sometimes growth retardants are much more effective than exogenous cytokinin application in delaying senescence (Weaver, 1972), and retardant-induced deferral of senescence of isolated leaf segments (Beever and Guernsey, 1967; Kessler et al., 1967; Knypl, 1967; Harada,

1968) or of intact plants (Appleby *et al.*, 1966; Halevy and Shilo, 1970; Guardia *et al.*, 1974; Orchard and Lovitt, 1976) has been widely reported. Again, dikegulac-induced enhancement of growth promoter and inhibition of growth inhibitor levels at preharvest stage of sunflower is also known (Bhattacharjee, 1984). Thus from reported observations it may be likely that dikegulac triggered the delaying of senescence of attached leaves by manipulating the hormonal level, particularly of cytokinin at later stages of plant growth, (Purohit and Chandra, 1983).

### **Lodging Behaviour and Yield**

Serious impairment of yield of many monocarpic crops often occurs as a result of lodging, specially when such crops are grown in extremely fertile soils and they attain a certain height with the subdued mechanical strength of stem. As dikegulac is a potent height shortening agent of plants, the efficacy of this chemical on alleviation of this handicap was tested by Bhattacharjee (1984) on a tall and a dwarf sunflower cultivar during monsoon period when maximum incidence of lodging occurs. Results showed that dikegulac regardless of its concentrations, reduced the severity of head lodging in both the cultivars. The tall cultivar was found to be extremely susceptible to lodging and dikegulac was most successful on this variety with respect to arrestation of lodging and consequently of improvement of the yield attributes like per cent 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 long phase of plant growth i.e. at preheading stage, and no such effect was apparent when dikegulac application was made at seed and seedling stage of the sunflower cultivars. Working on 3 cultivars of *Capsicum annuum* (Sandia, Espanol 1 and NM<sub>6</sub>), Mattia (1983) reported that though dikegulac application increased branching in all the 3 cultivars, it decreased yield in all of them, and dikegulac at 104 ml per plant resulted in no yield. Menhenett and Hanks (1983) showed that dikegulac was most effective chemical with respect to restriction of post-flowering extension growth of late season-tulip and checked the lodging tendency. But as to its role on qualitative flower dikegulac was proved most unsuitable because it increased flower bud blasting and gave rise to abnormally coloured perianth segments.

Effect of dikegulac on the alleviation of and consequent enhancement of yield can be explained by its effect on suppression of apical dominance (Bocion *et al.*, 1975) rendering plants short statured and on production of profuse mechanical tissues in stem (Bhattacharee and Gupta, 1984a) and enhancement of lignin as well (Bhattacharjee, 1984) strengthening the stem of sunflower. Such a role of dikegulac is suggestive of the fact that whatever might be its effect on direct modification of crop yield, the chemical may enhance yield atleast in those crops where reduction of yield occurs due to incidence of lodging.

### **Sprouting of Tubers**

A comparative study of the effect of dikegulac and GA<sub>3</sub> on potato sprouting reveal that dikegulac (25 to 400 µg/ml) delayed sprouting emergence and inhibits further growth of shoot while GA<sub>3</sub> has hastened the same (Purohit, unpublished data). Such delay caused by dikegulac may be due to antagonistic action of this chemical on endogenous GA level which consequently inhibits growth activities (sprouting) induced by GA<sub>3</sub>.

### **Callus Growth**

Promotion of callus growth by dikegulac at 10<sup>-6</sup> M in *Lycopersicum esculentum* was observed by Bocion and deSilva (1977a). Higher concentration of dikegulac (10<sup>-3</sup>M) in culture medium caused death of the callus. Such inhibitory effects of dikegulac at 10<sup>-3</sup>M could only be counteracted by similar concentration of GA<sub>3</sub>. When dikegulac (10<sup>-6</sup>M) was used in combination with GA<sub>3</sub> (10<sup>-7</sup> or 10<sup>-6</sup>M) the growth was found to be stimulated than that of GA<sub>3</sub> alone. These results therefore, suggests that dikegulac and GA<sub>3</sub> when used in combination, the effect remains additive while it is antagonistic at higher concentrations.

### **Summary and Conclusion**

The foregoing review thus clearly indicates that since the discovery and establishment of dikegulac as a potent growth regulator in 1975 by Bocion *et al.*, a comprehensive work in diverse fields of research has been accumulated through two

decades and half. From the numerous experimental results available to date, a light could be thrown on the problems and prospects of this novel growth regulator, and some new and promising approaches of research problems could also be designed out of these pioneering works. On the basis of available literature, the promising roles of dikegulac enabling to open commercially prospective research avenues are briefed, and a few possible suggestions of its fruitful utilization have been mentioned (Bhattacharjee *et al.*, 1986).

1. Dikegulac may be commercially exploited for maintaining seed vigour and viability, and thus the practice of conventional methods of seed storing may be improved with this chemical with a view to delaying storage deterioration which poses a serious problem to the crop growers.
2. As dikegulac shows encouraging results with respect to rendering plants tolerant towards adverse environmental stresses, the commercial feasibility of exploiting such an effect of dikegulac could be tested on a wide range of crop plants and be suitably utilized for agricultural practices.
3. Property of chemical pinching and consequent production of profuse axillary branches of dikegulac is promising for horticulturists and florists.
4. Dikegulac seems to be promising for monocarpic plants like sunflower, safflower etc. because the chemical, if applied at proper stage of plant growth, can produce some alterations in growth and metabolism of plants which are conducive to yield improvement. In fact, short-statured plants, enhanced mechanical strength in stem, deferment of senescence and prolongation of seed filling period are general characteristics of such treatments which are encouraging for monocarpic plants like sunflower and safflower.
5. Dikegulac-induced improved plant vigour and higher potential, particularly at later stages of sunflower growth could be efficiently exploited through hormonal application at the active seed-filling period. Such a strategy of yield improvement in sunflower, by treatments of dikegulac followed by hormones, may be tested in some other monocarpic crops.

6. Dikegulac may be potentially used on those crops which often prone to lodging resulting in serious loss of yield.

All such achievements appeared to be encouraging for agricultural and horticultural practices in addition to the academic interest of dikegulac. Hence, testing the commercial feasibility of dikegulac in conjunction with some other hormones in raising ideotype plant for higher productivity may be an attractive proposition. However, the criteria like selection of the optimum stage of application, concentrations of the chemical, exploitation of the imposed plant vigour through hormonal manipulation at the critical period of grain-filling etc. are the important determinants for obtaining covetable result.



# *Introduction*

## INTRODUCTION

*Sechium edule* Swartz (family : Cucurbitaceae, tribe : Scyvoideae) commonly known as chayote (English), Iskoos (Nepali), Quash (Bengali), is one of the important vegetable crops for the people in the hilly regions of the Darjeeling district of West Bengal. State in India (Mukhia *et al.*, 1982, Dey and Jana, 1988). It is also grown profusely in tropical America (Cook, 1901). It grows both in cultivated and wild conditions and flourishes in altitudes ranging from 500m to 2000m around Darjeeling hills. This hilly plant species is perennial; the above ground part die in winter but the tuberous roots thrive and new sprouts arise in the next rainy season. Fruiting time is from September to December. Tuberous roots, fruits as well as young leafy shoots of the plant are edible and are used as delicious table items. In recent years, this species has attracted cultivators as well as researchers because it seems to be highly dietary vegetable crop requiring no cumbersome field management and for its scope for developing ideal plant type for higher productivity by scientific manipulation, (Lama, 1988; Dolui and Jana, 1989; Shil, 1990; Lama, 2000).

The crop has a lot of plus points for its enthusiastic acceptance by the local cultivators of Darjeeling district as an ideal vegetable crop. These are : (1) minimum cost of maintenance in the field, (2), less susceptibility towards diseases, (3) higher productivity in the agroclimatic conditions of Darjeeling hills, (4) higher responsiveness towards organic manures and vigorous growth of the plant even in fallow land, (5) considerable food value of all its parts, (6) Strong storage potential of fruits and tuberous roots under ambient climatic conditions of storage prevailing in Darjeeling, (7) significant resistance capacity towards various climatic hazards, <sup>particularly</sup> against high relative humidity and biotic hazards particularly against fungi and bacteria (Lama, 2000).

Considering the prospects of cultivation of *Sechium edule* in Darjeeling hills, an attempt was made in the present investigation to produce an ideotypic plant having higher productivity by chemical manipulative agents, using plant growth regulators. Like other cucurbitaceous plants, there are some problems as to the higher productivity of chayote plants. Firstly, unwanted excess plant vigour and strong apical dominance, particularly during log phase of growth, cause impairment of crop yield. Secondly, in some varieties higher (even 50 : 1, Lama, 2000) ratio of male to female flowers causes lower yield of fruits, as male flowers function as pollen donors and are ineffective with respect to increasing fruit numbers. Thirdly, early senescence of the contributory leaves results in reduced supply of assimilates to the active reproductive sinks and the consequence is the smaller size of fruits and reduction of total yield. Fourthly, attack by some animal pests on the foliage, inflorescence and stem of the plant particularly during assimilate filling phase, renders the growing fruits less saturated or unsaturated sinks thereby resulting in smaller size of fruits and consequent impairment of crop yield. Even the life cycle of the plant is terminated shortly, and in case of severe infection yield becomes negligible or nil. Lastly, yield of the underground tuberous root is often reduced by some animal pests and soil nematodes which hinder tuberization process. Considering the above-mentioned problems of chayote cultivation, attempts were made to obviate or to reduce the degree of some of these deleterious features of the plant by chemical manipulative methods using plant growth regulators (one retardant class (Atrinal) and two promoter class (GA<sub>3</sub> and kinetin). To get rid of the undesired profuse vegetative growth, the selected growth retardant Atrinal is supposed to act the preferred instrument. There are ample reports in the literature that a number of growth retarding chemicals including the present one can successfully check unwanted excess plant vigour causing subdued plant growth (Knypl, 1979; Monselise, 1974; Bhattacharjee, 1984. The efficacy of the retardant on lowering plant vigour was determined by analysing the growth and metabolic status of the chayote plant. An attempt was made to increase the number of female flowers per plant by foliar application of kinetin, GA<sub>3</sub> and

IAA which are supposed to have some role on flowering as well as on sex expression in many cucurbits (Leopold and Kriedemann, 1975; Ghosh and Basu, 1982, 1983). The deleterious feature of the onset of earlier senescence of the active assimilate transporter leaves, was tried to overcome by using senescence deferral agents like kinetin and Atrinal which is reported to keep the foliage green even before preharvest stage of many monocarpic crops (Bocion et al., 1975, Nooden and Leopold, 1978; Bhattacharjee, 1984). The efficacy of these chemical manipulants was analysed through several established and reliable physiological and biochemical senescence evaluation indices. Thus, the prime objective of the present investigation was to obtain an ideotypic chayote plant <sup>for higher productivity</sup> after possible obviolation of the deleterious features, as mentioned, by chemical manipulative methods.



*Materials and  
Methods*

## MATERIALS AND METHODS

### **Plant material :**

Experiments of this investigation were performed with a promising vegetable crop chayote (*Sechium edule* Sw.), a squash like climbing plant of the family Cucurbitaceae under the tribe Sicyoideae. The plants are luxuriantly grown in Darjeeling hills of Eastern Himalayas at altitudinal ranges of 500 to 2500 meters (M) under cultivated as well as wild condition. After varietal screening of the species it was found that the varietal type available in Mirik (1850M) was superior to others growing in Sukhia Pokhri (1900M) and Darjeeling Town (2134 M) with respect to general vigour and yield of fruits (Vide Table 3). Detailed experiments were carried out with a specific varietal type (I, Vide Table 1) growing in and around Darjeeling town.

Chayote is a perennial species; the above ground parts die in winter but the tuberous roots thrive and new sprouts arise from the tuberous roots in the following season. The above ground part of the plant is monocarpic in nature and survives more than five months. Vegetative phase continues more than two months and fruiting phase persists for three months. Varieties growing in various altitudes of Darjeeling hills were recorded and altogether ten varietal types were screened and named alphabetically (A,B, C,D, E,F,G, H, I and J) mainly on the basis of morphological characters of fruits (Table 1). Important events during the life cycle of the plant were determined from the average data of four planting seasons i.e., 1992, 1993, 1994 and 1995 (Vide Table 2 and also Diagram 1). The underground tuberous part is perennial, enriched with carbohydrates and is highly potential for giving new plants under favourable situations.

### **Soil preparation and method of sowing :**

The whole experimental plot was divided in subplots each having an area of 3x3 M for raising plants of different treatments. The main plot was ploughed 3-4 times, cowdung and organic composts were amended with the soil before sowing the fruits, the main

propagating unit of this species. The <sup>sprouted</sup> ~~sprouted~~ mature healthy fruits were sown at a distance of 1.5M (plant-to-plant as well as row-to-row).

Owing to delay of field emergence, possibly because of occurrence of some inhibitors in fruit flesh, the mode of sowing was of two different kinds. Sowing of intact fruits and sowing of defleshed fruits. Sprouted fruits (intact or defleshed) were sown at the depth of 15 cm into the soil in a slight oblique manner placing the sprouted part towards the upperside and the propagules were watered at five-day intervals until seedlings developed.

### **Meteorological Data :**

Meteorological data viz., temperature, relative humidity and rainfall during the experimental years of 1992 to 1995 were procured monthwise from the Principal Office of Agriculture, Govt. of West Bengal, Darjeeling. The data were incorporated in Tables 4,5,6 and 7.

### **Design of Experiments :**

In this investigation experiments were designed under the following directions to analyse the effects of an agrihorticulturally promising and less explored growth retarding chemical sodium dikegulac (Na-dikegulac) or Atrinal (2,3:4-6-di-O-isopropylidene- $\alpha$ -L-xylo-2 hexalofuranosate) with a view to obtaining higher productivity. Keeping in mind some beneficial effects of raising chayote plants from defleshed fruits, with respect to quicker field emergence and maintaining higher plant potential (Lama 1990, personal communication), plants were developed from both intact fruits and defleshed fruits.

1. Analyses of the effects of fruit (intact or defleshed) treatment with Atrinal on growth, metabolism and crop yield (Section 1).
2. Analyses of the effects of Atrinal treated at sapling stage on growth, metabolism and crop yield (Section 2).

3. Analyses of the effects of Atrinal treated at preflowering stage on growth, metabolism and crop yield (Section 3).
  4. Analyses of the effects of Atrinal treated at preflowering stage followed by GA<sub>3</sub> and kinetin application at flowering stage on growth, metabolism and crop yield (Section 4).
- 1. Analyses of the effects of fruit (intact or defleshed) treatment with Atrinal on growth, metabolism and crop yield :**

A screening experiment was initially performed to observe the effects of quantum of fruit flesh removal (25%, 50%, 75% and 100%) for better development of chayote plants. It was found that plants raised from 50% defleshed fruits stand well in the field (data not shown) and hence experiments were done with 50% defleshed fruits (hereinafter called defleshed fruits) or intact fruits.

Healthy, sprouted fruits (intact or defleshed) of chayote were thoroughly sterilized with 0.1% HgCl<sub>2</sub> for 90 seconds and then air-dried. Subsequently, the fruits were presoaked with aqueous solutions of Atrinal (500, 1000 and 2000 µg/ml) for 24 hours and then sown in the experimental field in the month of February. Fruits (intact or defleshed) treated with distilled water for 24 hours served as the control set. From the field grown plants phenological, growth, biochemical and yield data were recorded.

#### **Phenological analysis :**

Important events during the life cycle of chayote plant were determined from the average data recorded in four planting seasons of the years 1992, 1993, 1994 and 1995 (Table 2 and also Diagram 1). The phases recorded include : field emergence phase, first leaf emergence phase, seedling phase, sapling phase, flower initiation phase, fruit formation phase, senescence phase and death phase.

**Growth analyses :**

For study of growth attributes of the plants raised from pretreated fruits (intact or defleshed) investigation was carried out from the date of sowing with the first field emergence as the initial phase of life cycle. Growth parameters like vine length and stem circumference (cm) of plants were recorded from each plot-grown plants of different ages (days, d) at specific stages viz., seedling stage (20-d), sapling stage (40-d), preflowering stage (60-d), fruiting stage (80-d) and senile stage (140-d). Data were recorded from the mean values of 5 uniformly grown plants, developed from the 5 uniformly sprouted fruits for each treatment. Stem circumference was recorded from the 10<sup>th</sup>, 11<sup>th</sup> and 12<sup>th</sup> internods of each plant. Further, the data on number of days (plant age) required for the inception of leaf senescence of plants were recorded and the results were represented with yield data like fruit number fruit weight and tuberous root per plant.

**Biochemical analyses :**

Biochemical analyses were carried out taking samples from leaves at five important phases of chayote plants viz., seedling phase, sapling phase, preflowering phase, fruiting phase and senile phase which correspond to 20-, 40-, 60-, 80- and 140-days of plant age respectively.

**Chlorophyll :**

Leaf tissues (100 mg) of each treatment were immersed in 5 ml methanol in test tubes and kept in freeze for 48 hours. The supernatant was decanted off and leaf samples were rinsed repeatedly with a little volume of methanol until they were completely free from green colour. Thus, the final volume of methanol was made to 10 ml and the intensity of the green colour was measured at 650 nm in spectrophotometer. The chlorophyll content was estimated following Arnon's (1949) principle.

**Protein :**

The chlorophyll free above leaf samples were crushed with 80% ethanol and centrifuged at 6000 g for 10 min. to make the pellet free from phenol. It was washed successively with 10% (w/v) cold trichloroacetic acid TCA, twice), ethanol (once), ethyl alcohol; chloroform (3:1, v/v, once), and finally with solvent ether as per the method of Kar and Mishra (1976). The pellet was then evaporated to dryness. The protein was solubilised by treating with 0.5 N NaOH at 80°C for 1 h. A definite volume (4 ml) was made with the extraction medium. It was then estimated by reacting the protein solution with Folin-phenol reagent and measuring the O.D. values spectrophotometrically at 650 nm according to the method of Lowry *et al.* (1951). The quantitative determination was made by comparing the O.D. values of a standard curve previously prepared using bovine serum albumin (BSA, Fraction-V powder, Sigma Chemical Co., USA).

**Carbohydrates :**

Carbohydrate levels (both soluble and insoluble fractions) were determined following essentially the method of McCready *et al* (1950) with minor modifications. Fifty mg leaf samples of each treatment were homogenized with boiling 80% ethanol, and centrifuged at 6000 g for 15 min. The supernatant was taken in a watch glass. This was repeated thrice, and the pooled supernatant was then evaporated to dryness. Trace of chlorophyll, if any, adhering on the surface of the watch glass were carefully removed using solvent ether. The remaining material in watch glass was taken in test tubes by washing them several times with 80% ethanol and the volume was made up to 10 ml. This was kept as a source of soluble carbohydrate. For the analysis of insoluble carbohydrate, the residue after centrifugation of the sample was digested with 5 ml 25% H<sub>2</sub>SO<sub>4</sub> at 80°C in a water-bath for 30 min. The extracted material after suitable dilution was taken as a source of insoluble carbohydrate.

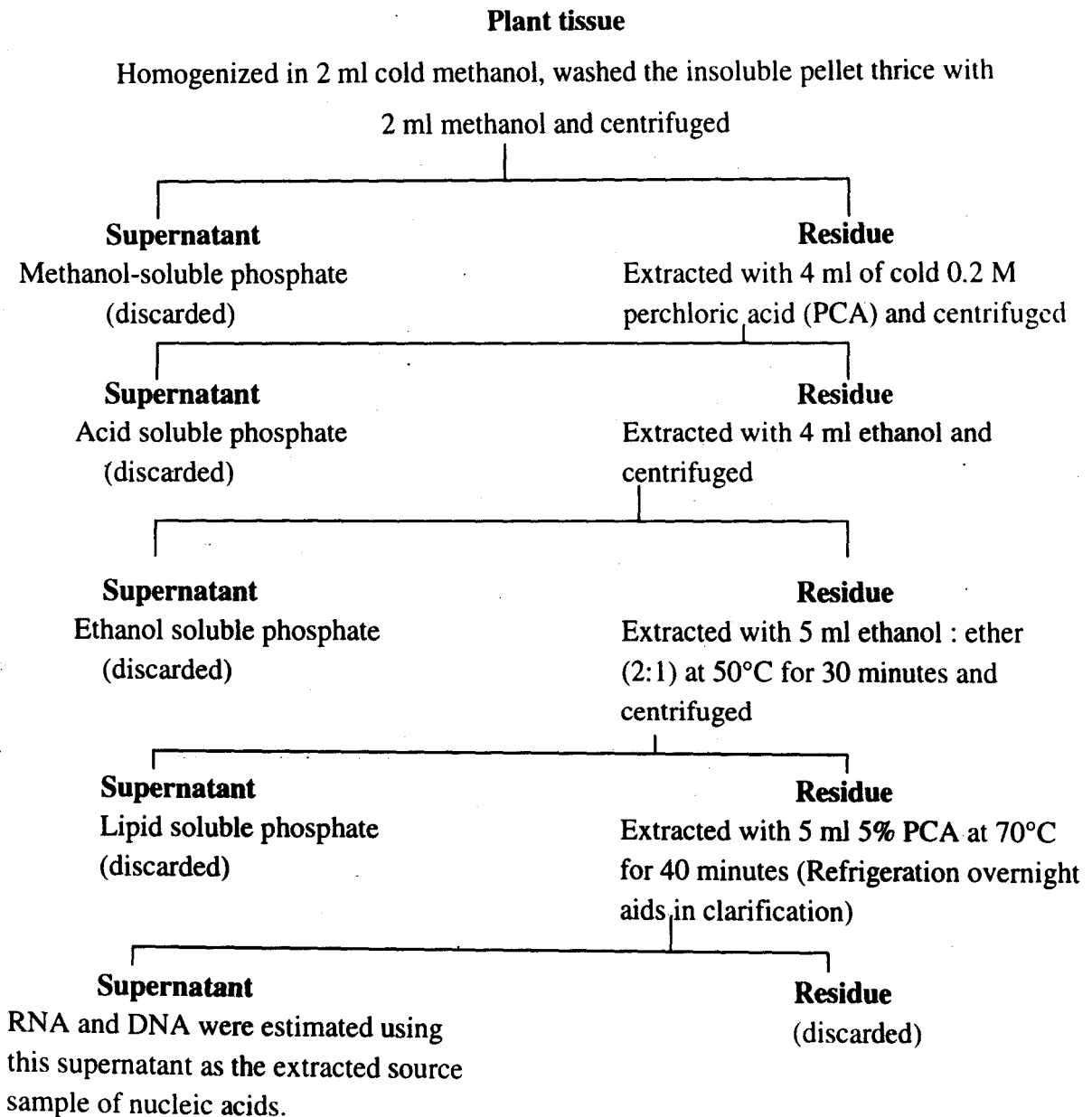
For quantitative measurement of both the carbohydrate fractions, 1 ml of the source sample from each was taken in a test tube and to it added 4 ml freshly prepared, precooled 0.2% anthrone reagent (200 mg anthrone in 100 ml concentrated analar H<sub>2</sub>SO<sub>4</sub>). After 15

minutes, the intensity of green colour was measured spectrophotometrically at 610 nm. Actual contents were determined from the standard curve with glucose.

### Nucleic acids :

Extraction of nucleic acids (both RNA and DNA) was made from 100 mg leaf samples of each treatment following the method of Cherry (1962), and the estimation was done as per the method of Markham (1955) modified by Choudhuri and Chatterjee (1970).

Extraction process of RNA and DNA was done as outlined below :



For the estimation of RNA 3 ml diluted extract (in 5% perchloric acid) in test tube was treated with an equal volume of freshly prepared orcinol reagent (1 g AR grade orcinol dissolved in 100 ml concentrated HCl containing 100 mg 0.1%  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ), and boiled in a water bath for 20 min. with glass marble at the mouth of the test tube. The mixture was then cooled, and the intensity of the blue green colour was measured at 700 nm. The blank used contained a mixture of 3 ml distilled water and 3 ml orcinol reagent, which were treated in an identical manner. RNA level was calculated from O.D. values from a standard curve prepared with yeast RNA.

For the estimation of DNA 1 ml of nucleic acid extract in a test tube was mixed with 5 ml freshly prepared diphenyl amine reagent (100 ml glacial acetic acid BDH. AR+ 2.7 ml  $\text{H}_2\text{SO}_4$  + 1 g AR grade diphenyl amine. The mixture was boiled in a water bath for 30 min with a glass marble at the top. After cooling, intensity of bluish colour was measured at 610 nm in the spectrophotometer. DNA level was quantified from the O.D. values of a standard curve prepared with herring sperm DNA.

#### **Catalase activity :**

Leaf tissues (500 mg) of each treatment were homogenized with 8 ml of chilled 0.1 M phosphate ( $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ ) buffer (pH 6.8). The homogenate was centrifuged in the cold at 3000 g for 15 min followed by 10,000 g for 20 min. the volume of the supernatant was made up to 10 ml with the same buffer, and this was used as crude enzyme source. The activity of the enzyme catalase was assayed following the method of Snell and Snell (1971) modified by Biswas and Choudhuri (1978). The reaction mixture for catalase consisted of 1 ml of the above extract and 1 ml of  $\text{H}_2\text{O}_2$  (0.005 M), incubated together at 37°C for 2 min. The reaction was stopped by adding 2 ml 0.1% titanil sulphate in 25%  $\text{H}_2\text{SO}_4$  (V/V), and the mixture was centrifuged at 6000 g for 15 min. The intensity of yellow colour was measured at 420 nm. The blank was prepared by inactivating the enzyme with the addition of titanil sulphate prior to  $\text{H}_2\text{O}_2$  addition.

**Peroxidase :**

Leaf tissues (500 mg) of each treatment were homogenized in cold 0.05 M sodium phosphate buffer (pH 6.5). The homogenate was centrifuged at 10,000 g for 10 min. The filtrate was used as enzyme source.

Activity of this enzyme was assayed following the method of Kar and Mishra (1976) with slight modification. Five ml of the assay mixture containing 300  $\mu$ M of sodium phosphate buffer (pH 6.8), 50  $\mu$ M catechol, 50  $\mu$ M  $H_2O_2$  and 1 ml of crude enzyme extract. After incubation at 25°C for 5 min, the reaction was stopped with the addition of 1 ml of 10%  $H_2SO_4$ . The colour was read at 430 nm. In case of blank sample, the enzyme was first inactivated with the addition of  $H_2SO_4$ .

**IAA-oxidase activity :**

Extraction of this enzyme was made from 100 mg leaf tissue with 12ml of cold 0.2M sodium phosphate (pH 6.1). The activity of IAA oxidase was assayed following the method of Gordon and Weber (1951) as modified by Ramadas (1968). The reaction mixture contained 1ml (1mM) of 2,4-dichlorophenol, 1ml of MnCl 1mM, 0.6ml of 0.03M sodium citrate buffer (pH 4.5) and 1ml of enzyme extract. This was incubated for 50 min at room temperature and then the reaction was stopped by pouring 1ml of 20%  $HClO_4$  to the mixture. One ml of the assay mixture was reacted with 3ml of Salkowski reagent (50 ml of 35%  $HClO_4$  + 1ml 0.5 N  $FeCl_3$ ), and the reading was taken at 525 nm in the spectrophotometer.

**RNAse activity :**

Fresh leaves (100 mg) were homogenized with 5ml of 0.1M sodium phosphate buffer (pH 6.4) at 0°C and centrifuged at 10,000g for 20 min. The supernatant was made up to 10ml with the same buffer solution and this was used as crude enzyme source. Assay was made as per the method described by Biswas and Choudhuri (1978).

The reaction mixture for RNAse consisted of 1ml of the enzyme extract and 1ml of yeast RNA (1mg/ml) dissolved in 0.1M sodium phosphate buffer (pH 5.7). The mixture was then

incubated for 30min at 37°C, and the reaction was stopped by adding 0.2ml perchloric acid (70%). After centrifugation at 6000g, the supernatant was mixed with 5ml of BSA (0.5µg/ml) dissolved in 0.1M sodium acetate buffer (pH 4.0). After 5 min the turbidity developed was stabilised with 2ml of 0.1% gelatine and measured at 420 nm. Activity of this enzyme was expressed following the principle of Fick and Qualset (1975).

In each case of enzyme assay, value at zero time was taken as blank, and the activity of each enzyme was expressed as  $(\Delta A \times T_v) / (t \times v)$ , where  $\Delta A$  is the absorbance of the sample after incubation minus the absorbance at zero time.  $T_v$  is the total volume of the filtrate,  $t$  is the time (min) of incubation with substrate and  $v$  is the total volume of the filtrate taken for incubation (Bhattacharjee, 1984). The activity of each enzyme was expressed as unit/ fresh weight/h.

#### **Yield analyses :**

After senile stage yield data were analysed in terms of the production of fruits and underground tuberous roots and yield attributes recorded include : total number of mature fruits per plant, total weight (kg) of fruits per plant and total weight (kg) of tuberous roots per plant.

## **2. Analyses of the Effects of Atrinal treated at Sapling Stage on Growth, Metabolism and Crop Yield.**

Foliar application with aqueous solutions of Atrinal (500, 1000 and 2000 µg/ml) containing Teepol (surfactant) were given at around 9 am each day for three consecutive days on thirty-day-old field grown saplings raised from intact or defleshed fruits. Saplings treated with distilled water served as control set. From the field grown plants growth, biochemical and yield data were recorded.

#### **Growth analyses :**

After foliar application with Atrinal growth data, measured in terms of vine length and stem circumference, were recorded at four developmental stages of the plant viz.,

sapling stage, preflowering stage, fruiting stage and senile stage which correspond to 40-, 60-, 80- and 140-days of plant age respectively. Like fruit treatment (intact or defleshed) data were analysed from the mean values of 5 uniformly growing plants, and here also plant age (days) to inception of leaf senescence was recorded.

#### **Biochemical analyses :**

For biochemical analyses samples were taken from leaves of plants, which experienced foliar treatment with Atrinal or distilled water. Methods for extraction and estimation of biochemical parameters like chlorophyll, protein, carbohydrate, nucleic acids (RNA and DNA), catalase, peroxidase, RNase and IAA-oxidase were described earlier in section 1 (Fruit treatment).

#### **Yield analyses :**

Yield attributes analysed are the same as in Section 1 i.e., total number of fruits per plant as well as total fruit weight (kg) and tuberous root weight (kg) per plant.

### **3. Analyses of the Effects of Atrinal Treated at Preflowering Stage on Growth, Metabolism and Crop Yield :**

Sixty-day-old field grown plants, raised from intact and defleshed fruits, were foliarly sprayed with Atrinal (500, 1000 and 2000  $\mu\text{g/ml}$ ) or distilled water containing Teepol (surfactant) at around 9 am each day for three consecutive days. From the field grown plants growth, biochemical and yield data were recorded.

#### **Growth analyses :**

Growth data recorded in this experiment were the same as done in case of fruit (intact or defleshed) and sapling treatments mentioned in Section 1 and 2 respectively. Here data were recorded at two stages of plant development i.e., fruiting stage and senile stage, which correspond to 80- and 140- days of plant age respectively.

**Biochemical analyses :**

Biochemical analyses were done taking samples from leaves of the plants, raised from intact and defleshed fruits, at fruiting and senile stages of plant development. The parameters analysed were the same as done in case of fruit and sapling treatments and the methods of extraction and estimation were described in Section 1.

**Yield analyses :**

Yield attributes recorded in this experiment are the same as done in Sections 1 and 2.

#### **4. Analyses of the Effects of Atrinal Treated at Preflowering Stage Followed by GA<sub>3</sub> and Kinetin Application at Flowering Stage on Growth Metabolism and Yield.**

In this experiment, foliar treatment with Atrinal (1000 µg/ml) or distilled water containing Teepol was given the preflowering state of sixty-day-old field grown plants, raised from intact and defleshed fruits, for three consecutive days. Such plants were subsequently sprayed with GA<sub>3</sub> (100 µg/ml) or kinetin (100 µg/ml) for further three consecutive days starting from 70-day-old plants. Growth, biochemical and yield data were recorded like previous experiments. In addition, here total number of male and female flowers per plant was counted. Considering the most effective concentration of Atrinal (1000 µg/ml), in this experiment only single dose of Atrinal was used.

**Growth analyses :**

Like previous experiments as mentioned in Sections 1,2 and 3 growth data were recorded from plants raised from intact and defleshed fruits. And data on vine length and stem circumference were taken at fruiting and senile stages as in experiments mentioned in Section 3.

**Biochemical Analyses :**

Biochemical parameters and methodologies of analyses were the same as done in Section 1. Here samples were taken from leaves of plants which experienced foliar treatment with Atrinal (1000 µg/ml) at the preflowering stage or from plants which received Atrinal

treatment at preflowering stage followed by GA<sub>3</sub> and kinetin (100 µg/ml each) treatment at flowering stage.

#### **Yield Analyses :**

Yield data recorded in this experiment are the same as done in previous experiments. Here in addition to the effect of Atrinal (1000 µg/ml) effects of supplementary treatments (GA<sub>3</sub> and kinetin) on modifying crop yield was analysed.

#### **Statistical Analyses :**

All the growth, biochemical and yield data recorded in this investigation, were statistically analysed at the treatment and replication levels. In the tables LSD (least significant difference) values at 95% confidence limits were incorporated (Panse and Sukhatme, 1967).

# *Results*

## RESULTS

Table 1 shows varietal differences of *Sechium edule* growing in various altitudes of Darjeeling hills. Altogether 10 varietal types have been established on the basis of morphological characters on mature fruits viz., length, breadth, girth, weight and colour of fruits as well as on the basis of density, length and mode of distribution of hairs on the surface of fruits. Among the categories the types A,B, and J produce hairless fruits and the type H produce fruits with scanty hairs. Other varietal types yield fruits with hairs of specific nature and they show specific mode of distribution on fruit surface.

Important events during the life cycle of the experimental chayote plant have been depicted in Table 2 and Diagram 1. The leafy above ground part of chayote is monocarpic in nature and the data reveal that the plant survives more than 5 months with prolonged duration of log phase and stationary phase of growth. The underground tuberous part is perennial and survives for several years. Meteorological data at the experimental station (Darjeeling) during the experimental years of 1992, 1993, 1994 and 1995 (monthwise) are represented in Table 4, 5 6 and 7 respectively. As planting of chayote starts from late February maximum vegetative growth takes place during the months of April, May and June, and harvesting of fruits is completed by October each year, it seems apparent that during plantation comparatively low temperature, low relative humidity and low rainfall are ideal climatic factors. Vigorous plant growth and fruit maturation takes place during the monsoon months of June and July when maximum rainfall, fairly high relative humidity and moderate temperature are recorded.

While undertaking extensive survey programme of different altitudinal areas of Darjeeling hills three distinct chayote growing localities at different altitudes were identified. There are Mirik (1850 M), Sukhia Pokhi (1900M) and Darjeeling Town (2134M). Plants are distinctly different with respect to their vegetative (vine length, leaf number), reproductive (female and male flowers) and yield (fruit weight) characters (Table 3). Data showed that the varietal type growing in Mirik was superior in all respects, particularly on yield of fruits.

## **Analyses of the Effects of Fruit (intact or defleshed) Treatment with Atrinal on Growth, Metabolism and Crop Yield**

### **Changes in chlorophyll and protein Levels (Table 8) :**

Results showed that pretreatment of both intact and defleshed fruits with Atrinal increased chlorophyll and protein contents in leaves of chayote plants when data were recorded at seedling and sapling stages of plant development. However, subsequent changes observed at preflowering, fruiting and senile stages were statistically insignificant. Data further revealed that chlorophyll and protein levels were comparatively high in leaves of plants raised from defleshed fruits. Among the three concentrations of Atrinal used 1000  $\mu\text{g/ml}$  was found to be most effective in this regard.

### **Changes in soluble and insoluble carbohydrate levels (Table 9) :**

Pretreatment of the fruits (both intact and defleshed) with Atrinal, irrespective of its concentration, resulted in significant increase of soluble and insoluble carbohydrate levels in leaves of chayote. However, such increase was found to be transient and did not persist beyond sapling stage of plant development. Here also, the carbohydrate levels were found higher in leaves of plants raised from defleshed fruits.

### **Changes in RNA and DNA levels (Table 10) :**

After a transient increase of DNA content at the seedling stage only Atrinal-induced changes of DNA levels failed to attain statistical significance at the subsequent sampling periods of sapling, preflowering, fruiting and senile stages. The changes of RNA level followed the same trend as found in the changes of DNA level but here Atrinal-induced promotive effect persisted up to sapling stage.

### **Changes in catalase and peroxidase activities (Table 11) :**

Activities of both catalase and peroxidase enzymes were stimulated in leaves as a result of pretreatment of intact and defleshed fruits with 500, 1000 and 2000  $\mu\text{g/ml}$  Atrinal. Here also, the chemical-induced stimulatory activities of the enzymes were not recorded<sup>at</sup> the preflowering, fruiting and senile stages of plant development. This table clearly revealed the

best response of Atrinal at 1000 µg/ml. Between the two different modes of plant development, plants raised from defleshed fruits showed higher activity of both the enzymes and such results are fairly uniform regardless of treatments at all the development stages.

#### **Changes in IAA-oxidase and RNase activities (Table 12) :**

Unlike the changes of catalase and peroxidase activities, IAA-oxidase and RNase activities were found significantly low in leaves of chayote plants, raised from Atrinal pretreated fruits (both intact and defleshed). However, the chemical-induced subdued activities of the enzymes were observed only at seedling and sapling stages of plant development and the changes recorded beyond sapling stage failed to attain statistical significance.

#### **Changes in vine length and stem circumference (Table 13) :**

Biochemical changes in leaves were found to be associated with the changes of growth parameters like vine length and stem circumference of chayote plant as a result of fruit pretreatment with the three different concentrations of Atrinal. Data revealed that vine length was significantly reduced by Atrinal and such effect was found to be strictly concentration dependent. On the other hand, stem circumference was found to increase in plants which were raised from the chemical pretreated fruits. However, Atrinal-induced retardation of vine length and enhancement of stem circumference was found to persist only up to sapling stage of plant development. Data also showed that plants raised from defleshed fruits were apparently superior to the plants raised from intact fruits.

#### **Changes in number of days required for inception of plant senescence, fruit number, fruit weight and tuberous root-weight per plant (Table 14) :**

Atrinal-induced transient changes of growth and biochemical parameters did not alter yield attributes, recorded in terms of fruit number, fruit weight and tuberous root weight per plant as well as the time (days of plant age) for onset of plant senescence, determined by observing the yellowing of leaves. The data inserted in this Table are fairly

uniform regardless of treatments as well as the modes of plant development and all the changes were noted to be statistically insignificant.

### **Analyses of the Effects of Atrinal Treated at Sapling Stage on Growth, Metabolism and Crop Yield**

#### **Changes in chlorophyll and protein levels (Table 15) :**

Foliar application with Atrinal (500, 1000 and 2000  $\mu\text{g/ml}$ ) for three consecutive days at the sapling stage of chayote plants, raised from both intact and defleshed fruits, resulted in significant reduction of chlorophyll and protein levels in leaves of all samples analysed at the sapling stage only. After a transient set back the chemical-treated plants were not only found to retain the normal levels of chlorophyll and protein but such levels were recorded to be even higher than control plants at least at the fruiting and senile stages of plant development. Such effects were found true in case of plant samples raised from both intact and defleshed fruits.

#### **Changes in Soluble and Insoluble Carbohydrate Levels (Table 16) :**

Almost an identical change like that of chlorophyll and protein, was observed when the Atrinal-induced changes of soluble and insoluble carbohydrate levels were analysed. Here also, the chemical exerted an inhibitory effect which was merely transient and recorded only at the sapling stage. The inhibitory effect of Atrinal was found to overcome at the subsequent stage (preflowering) of plant development. At fruiting and senile stages levels of both soluble and insoluble carbohydrate contents were found higher in the chemical treated samples.

#### **Changes in RNA and DNA levels (Table 17) :**

Atrinal-induced changes of RNA and DNA levels in chayote leaves were found distinctly inhibitory at the initial observation period i.e. at sapling stage. Such inhibitory action of the chemical was nullified at the preflowering stage, as the changes observed were not significant at all the concentrations of the chemical used. Interestingly, the chemical action was found promotive when data were recorded at the fruiting and senile stages of

plant development, and the effect of 1000  $\mu\text{g/ml}$  Atrinal treatment was found most efficient in this regard.

#### **Changes in catalase and peroxidase activities (Table 18) :**

Activities of both the enzymes were suppressed by all the concentrations of Atrinal when data were recorded at the sapling stage. Like other biochemical changes recorded earlier this retardation effect was erased quickly and at the fruiting and senile stages of plant development activities of the enzymes in Atrinal-treated samples were found higher than distilled water treated (control) samples. Here also, Atrinal 1000  $\mu\text{g/ml}$  showed the best response, and plants raised from defleshed fruits showed comparatively higher potential with respect to maintenance of higher activities of such enzymes.

#### **Changes in IAA-oxidase and RNase activities (Table 19) :**

Unlike the changes in the activities of catalase and peroxidase enzymes, a reverse trend of changes in that of IAA-oxidase and RNase were recorded. Data clearly revealed that all the concentrations of Atrinal more or less suppressed the activities of these enzymes after a transient increase of the enzyme activities at the sapling stage only.

#### **Changes in vine length and stem circumference (Table 20) :**

Biochemical changes were associated with the changes of growth parameters like vine length and stem circumference. Results showed that Atrinal significantly reduced the length of vine at all the concentrations used and this inhibitory effect was found to be maintained throughout the observation period's i.e., till senile phase. On the other hand, stem circumference was found to increase in all the plants which underwent foliar treatment with the three concentrations of Atrinal. The chemical-induced changes of vine length and stem circumference was found to be strictly concentration dependent and thus 2000  $\mu\text{g/ml}$  Atrinal was most effective for inhibiting vine length and enhancing stem circumference.

### **Changes in Number of Days Required for Inception of Plant Senescence, Fruit Number, Fruit Weight and Tuberos Root Weight Plant (Table 21) :**

At least at the two higher concentrations of Atrinal (1000 and 2000  $\mu\text{g/ml}$ ) delaying of senescence of chayote plants was recorded. The chemical was found to be ineffective for significant enhancement of fruit number at any of the concentrations used. However, plants raised from defleshed fruits showed higher fruit numbers than the plants raised from intact fruits. Fruit weight was significantly increased at the two lower concentrations (500 and 1000  $\mu\text{g/ml}$ ) and the root weight was found to increase significantly at the two higher concentrations (1000 and 2000  $\mu\text{g/ml}$ ) of Atrinal.

### **Analyses of the Effects of Atrinal Treated at Preflowering Stage on Growth, Metabolism and Crop Yield**

#### **Changes in chlorophyll and protein levels (Table 22) :**

Foliar treatment with Atrinal at the preflowering stage resulted in significant increase of chlorophyll and protein levels in leaves of plants raised either from intact fruits or defleshed fruits and such increase was found to persist till senile stage. Best response was recorded at 1000 $\mu\text{g/ml}$  Atrinal treatment.

#### **Changes in soluble and insoluble carbohydrate levels (Table 23) :**

Atrinal-treated plant samples significantly augmented the levels of soluble and insoluble carbohydrates in leaves of chayote plant. Here also, stimulatory activity was found most significant at 1000  $\mu\text{g/ml}$  Atrinal treatment.

#### **Changes in RNA and DNA levels (Table 24) :**

The changes of RNA and DNA levels in leaves as a result of foliar treatment with Atrinal was found to be identical with that of chlorophyll, protein and carbohydrates recorded in leaves of plants which underwent foliar treatment with Atrinal at the preflowering stage.

**Changes in catalase and peroxidase activities (Table 25) :**

Like sapling treatments, foliar treatment of chayote plants with the three concentrations of Atrinal at the preflowering stage efficiently enhanced the activities of both the enzymes and such effect was true both in case of plants raised from intact fruits or defleshed fruits.

**Changes in IAA-oxidase and RNase activities (Table 26) :**

A reverse picture was noted when the changes in the activities of IAA-oxidase and RNase enzymes were compared with that of peroxidase and catalase enzymes. Here, activities of both IAA-oxidase and RNase enzymes were found to decline significantly in the leaves of plants which received foliar treatment with Atrinal at the preflowering stage.

**Changes in vine length and stem circumference (Table 27) :**

Atrinal-induced changes of biochemical parameters were associated with remarkable change of vine length and stem circumference. Data revealed that the chemical, irrespective of its concentrations, significantly retarded vine length and enhanced stem circumference at both the observation periods i.e., fruiting stage and senile stage of plant development.

**Changes in Number of Days Required For Inception of Plant Senescence, Fruit Number, Fruit Weight and Tuberous Root Weight Per Plant (Table 28) :**

Atrinal (1000 and 2000  $\mu\text{g/ml}$ ) caused significant deferment of plant senescence in both the plant samples developed from intact or defleshed fruits. The low concentration (500  $\mu\text{g/ml}$ ) effect was found to be insignificant in this regard. However, the chemical at all its concentrations failed to induce any significant change with regard to the enhancement of total fruit numbers per plant. Fruit weight and tuberous root weight per plant was found to increase significantly at the two higher concentrations of Atrinal (1000 and 2000  $\mu\text{g/ml}$ ).

## **Analyses of the Effects of Atrinal Treated at Preflowering Stage Followed by GA<sub>3</sub> and Kinetin Application at Flowering Stage on Growth, Metabolism and Crop Yield**

### **Changes in chlorophyll and protein levels (Table 29) :**

Data clearly revealed that foliar treatment with Atrinal (1000 µg/ml) resulted in significant enhancement of chlorophyll and protein levels and such enhancing effect of Atrinal was augmented when the chemical treated plants were further treated with kinetin (100 µg/ml). However, the supplementary treatment with GA<sub>3</sub>, on Atrinal treated plants, was found to be ineffective and such effect was found true in case of plants raised from both intact and defleshed fruits.

### **Changes in soluble and insoluble carbohydrate levels (Table 30) :**

Here also, Atrinal-induced stimulatory effect on the levels soluble and insoluble carbohydrate was found additive when foliar treated plants with Atrinal (1000 µg/ml) at the preflowering stage was further experienced a foliar treatment with kinetin (100 µg/ml) at the flowering stage of plant development. GA<sub>3</sub>-induced effects (either on control or Atrinal-treated plants) were insignificant in this regard.

### **Changes in RNA and DNA levels (Table 31) :**

The results of supplementary treatments with GA<sub>3</sub> or kinetin at the flowering stage on Atrinal-pretreated plants were found to be identical with the changes of chlorophyll, protein and carbohydrate levels recorded from leaves of plants which underwent Atrinal followed by the hormonal treatments.

### **Changes in catalase and peroxidase activities (Table 32) :**

Results of this Table revealed the effective supplementary treatments i.e. kinetin treatment given at the flowering stage of the plants which were previously treated with 1000 µg/ml Atrinal. Such effect of kinetin was found true in both the plant samples raised from intact as well as defleshed fruits.

### **Changes in IAA-oxidase and RNase activities (Table 33) :**

Atrinal-induced retardation action of IAA-oxidase and RNase enzymes was augmented to a further degree when the chemical treated plants was further experienced foliar treatment with kinetin (100  $\mu\text{g/ml}$ ) only. Thus, the level of the enzymes in Atrinal plus kinetin treated sample was found to be even lower than Atrinal minus kinetin treated plant samples.

### **Changes in vine length and stem circumference (Table 34) :**

$\text{GA}_3$  significantly increased vine length and Atrinal-induced retardation of vine length was overcome by supplementary treatments with  $\text{GA}_3$ . Kinetin was found to be least effective or almost ineffective in this regard. On the other hand,  $\text{GA}_3$  failed to enhance stem circumference either singly or in combination with Atrinal treatments while kinetin effect was found to be slightly promotive in this regard.

### **Changes in number of days required for inception of plant senescence, fruit number, fruit weight and tuberous root weight per plant (Table 35) :**

Atrinal-induced deferral of plant senescence was found additive when plants received supplementary treatment with kinetin only. Data revealed that onset of senescence occurs at the plant age of 155 days in case of Atrinal-treated plants as against 142 days of control plants. And in Atrinal plus kinetin treated plants onset of senescence was delayed to a further extent (162 days of plant age). But fruit number per plant was found to increase significantly only in  $\text{GA}_3$ - treated samples either as single treatment or as follow-up treatment after Atrinal. Kinetin was found to be almost ineffective on enhancing fruit number. However, fruit weight per plant was found to increase both in  $\text{GA}_3$  and kinetin treatments either as single treatment or as supplementary treatment i.e. Atrinal +  $\text{GA}_3$  or Atrinal + kinetin.  $\text{GA}_3$ , however, failed to enhance tuberous root weight per plant.

### **Changes in the number of female and male flowers per plant (Table 36) :**

Results revealed that  $\text{GA}_3$  efficiently enhanced both female and male flowers per plant either as single treatment or as supplementary treatment (Atrinal 1000 +  $\text{GA}_3$ ) after pretreatment of the plants with Atrinal at the preflowering stage. However, kinetin effect

was found to be differential with respect to changes of two sexes of flower. Kinetin, particularly as supplementary treatment (Atrinal 1000 + kinetin 100), was effective for enhancement of female flowers per plant but kinetin-induced enhancement of male flowers was not found significant at least in case of plant samples which were raised from defleshed fruits.



# *Discussion*

## DISCUSSION

Influence of growth retarding chemicals on modification of growth, metabolism and yield have been extensively studied and a comprehensive report has been incorporated in an excellent review by Cathey (1964). Atrinal or sodium dikegulac, the key plant growth regulator used in this investigation, was first reported by Bocion *et al* (1975) and since then its effects on the changes of different aspects of growth and metabolism have been studied by a number of workers (Arzee *et al.*, 1977; Hield *et al.*, 1978; Bhattacharjee, 1984; Bhattacharjee *et al.*, 1986; Maity *et al.*, 2000). Keeping in mind a balanced growth retardation effect of this chemical particularly in case of plants having a strong apical dominance (Arzee *et al.*, 1977) like members of cucurbitaceae, an attempt was made to modulate plant growth and crop yield of an agrihorticulturally promising crop *Sechium edule* (chayote).

Many cucurbitaceous vegetable crop plants growing in India suffer from the drawback of having undesired excessive vegetative growth which often cause serious impairment of crop yield and thus in many occasions vigorous plant<sup>^ growth</sup> and higher productivity becomes inversely correlated (Shil, 1990). The present experimental plant chayote was also not found to be an exceptional species showing this generalized behaviour of cucurbits. Keeping in mind this prime negative yield attributing characters of chayote plant an attempt was made to increase crop yield simply by restraining the undue vigour using Atrinal which had been established as potent suppressor of vegetative growth of many agricultural and horticultural plants. We are now quite aware of the fact that yield impairment is resulted in many crop plants owing to their unwanted excess vigour which consequently deprive the reproductive sinks of their optimum need of assimilates (Weaver, 1972; Bhattacharjee *et al.*, 1984; Milthrope and Moorby, 1988).

After initial screening of varietal types and selecting of optimum concentration range of Atrinal suitable for this plant, applications were timed at three different developmental stages of the plant viz., sprouting stage of fruits, sapling stage and preflowering stage of the plant. The chemical induced changes of some growth and biochemical parameters were

analysed at different developmental stages of the plant and reflection of such changes on yield attributes were recorded. Again supplementary treatments with Atrinal followed by plant hormones like GA<sub>3</sub> and kinetin were given at the flowering stage of plant growth, and changes in modification of growth, metabolism and yield were analysed. Considering the beneficial effects of plant development from defleshed fruits, experimental plants were raised both from intact and defleshed fruits. The results obtained in this investigation were discussed at length from the available literature in this field and allied fields of research.

The present investigator and his associates after a thorough exploration in the chayote growing regions of Darjeeling hills, reported the existence of ten varietal types (Table 1) which somewhat differ among themselves with respect to their productivity, morphological characters of mature fruits, vine length and branching pattern (Lama *et al.*, 1994). Important events in the life cycle of this varietal type were analysed and found that vigorous vegetative growth starts around 50 days of plant age and continue up to active fruiting phase and this was determined as the log phase of plant growth (Table 2 and Diagram 1).

Results on intact on defleshed fruit treatment with Atrinal revealed that the chemical, irrespective of its concentrations, failed to induce any permanent effect on modification of growth, metabolism and yield of chayote plant. Data on biochemical changes in leaves showed that Atrinal-induced increase in chlorophyll and protein (Table 8), soluble and insoluble carbohydrate (Table 9), RNA and DNA (Table 10) levels were recorded at the seedling and sapling stages of plant development and subsequent changes were found to be statistically at par with that of control values. Likewise, activities of catalase-peroxidase enzymes (Table 11) remained high in the Atrinal-treated samples only up to sapling phase, and activities of the catabolic enzymes IAA-oxidase and RNase (Table 12) remained subdued up to sapling stage. Growth parameters like vine length was reduced and stem circumference was increased (Table 13) by the Atrinal treatments up to sapling stage and thereafter all changes were found insignificant.

Such results are indicative of the fact that Atrinal possibly hindered the biosynthetic processes of the macromolecules which actively occur at the early stage of plant

development, but with the progress of plant age the inhibitory effects were nullified because of diminished action of the retardant and consequent revival of the biosynthetic machinery of the cellular components. The resultant biochemical changes were correspondingly reflected in the plant growth as evident from the reduced vine length and enhanced stem circumference recorded at seedling and sapling stages only.

Reports exist in the literature that growth retardant temporarily exert inhibitory effects on seedling growth and metabolism (Knypl and Chylinska, 1972; Ben-Gad *et al.*, 1979; Bhattacharjee, 1984; Bhattacharjee *et al.*, 1986). Ben-Gad *et al.* (1979) observed that elongation of *Citrus* seedlings was initially retarded by SADH treatment but vigorous growth was resumed thereafter. Similar retardant-induced transient inhibition followed by rapid growth was also observed by Monselise *et al.* (1966), Sachs and Mock (1975), Bhattacharjee (1984) and many others who reported such effect using conventional retardants like CCC, SADH, 2,4-DNC, AMO 1618, MH morphactin etc, but reports with Atrinal are rather scanty.

There are reports in the literature that Atrinal affect seedling growth and metabolism of a good number of plant species (Bocion and De Silva, 1976; Arzee *et al.*, 1977; Purohit, 1979; 1980a,b,c; Bhattacharjee and Gupta 1981a,b; Bhattacharjee *et al.*, 1986). Arzee *et al.* (1977) showed that the overall seedling growth of zinnia, sunflower and chrysanthemum was affected with regard to the shortening of internodes, abnormal growth of leaves and disruption of apical dominance. Several reports revealed that Na-dikegulac reduced the seedling growth of sunflower (Purohit, 1979, 1980a,b), *Avena sativa* (Purohit and Chandra, 1980), *Brassica campestris* (Purohit, 1979, 1980c) or *Glycine max* (Purohit and Chandra, 1981) and the effect was found to be concentration dependent. Purohit (1979, 1980a,b) also reported that concomitant with the reduction of seedling growth, Atrinal adversely affected chlorophyll biosynthesis and reduced protein as well as sugar contents.

The results of this investigation was thus in conformity with the reported observations with Atrinal or some other growth retardants on some other plants. Initial retardation of growth and metabolism in chayote plant followed by alleviation of such

inhibitory effects might be explained considering the proposition made by Ben-Gad *et al.* (1979). From their experiments on the distribution of  $^{14}\text{C}$  labelled assimilates in SADH-treated plants, the authors concluded that assimilates and growth substances accumulated during the period of inhibition. Such accumulated materials and growth hormones were utilized fully during the subsequent periods when normal growth and metabolism and consequent rejuvenation of the plants resumed. In fact, such accumulation of growth substance during the periods of inhibition by growth retardants was also found in several studies (Frydman and Wareing, 1974; Kuo and Pharis, 1975; Fillipovich and Rowe, 1977). Arzee *et al.* (1977) using Atrinal showed that overall seedling growth of zinnia, sunflower and chrysanthemum was affected with regard to the shortening of internodes, abnormal growth of leaves, disruption of apical dominance, inhibition of DNA synthesis and chlorosis of leaves. In their investigation, the authors showed by whole plant autoradiography that the chemical moved towards acropetal direction and triggered its physiological action from the shoot-tips. They further reported that the adverse effects were later overcome and in all the three species convoluted and chlorotic leaves were regreened after a transient degreening.

The temporary inhibitory effect, as observed in the present study, as a result of fruit (intact or defleshed) treatment at the sprouting stage, might therefore be the effect of the growth retardant in the arrestation of the activities of overall biochemical machinery within the plant tissue and these cumulatively resulted in subdued plant metabolism and consequent shortening of vine length as well as increased radial growth of stem.

Results of Atrinal-induced fruit (intact or defleshed) treatment at the sprouting stage also revealed that yield components of chayote, recorded in terms of fruit number, fruit weight and tuberous root weight per plant as well as days for inception of plant senescence (Table 14) remained unchanged at all the concentrations of the chemical. This result can be substantiated from the normal behaviour of growth and metabolism as well as unchanged potential of the plants during flowering, fruiting and senile stages after a transient initial adverse effect. In fact, as yield components and senescence inception time were recorded at the advanced stage in the life cycle of the plant, the initial adverse effects of the retardant

were totally nullified leaving their no residual influence on modulating the yield components and senescence.

The present observation on the futile effect of Atrinal on modifying yield attributes can be corroborated from the findings of Lovett and Orchard (1976) who reported that CCC could augment yield alongwith inducing morphological and anatomical changes of sunflower when applied at log phase of plant growth only, and its application at lag phase was ineffective. Similar observation was recorded by Dorrel (1973). In sunflower Bhattacharjee (1984) reported that seed treatment or early seedling treatment with some growth retardants neither impaired or stimulated productivity. In the present study with chayote the results thus accord with reports of previous workers.

In the present investigation, results on foliar treatment with Atrinal at the sapling stage (30-day-old plants) revealed that the retardant-induced changes in chlorophyll and protein (Table 15), soluble and insoluble carbohydrate (Table 16), RNA and DNA (Table 17), levels as well as activities of catalase and peroxidase (Table 18) enzymes in leaves were inhibitory only at the initial observation period of 40-day-old plants. Such inhibitory effects were erased shortly, and the levels of the biochemical variables were higher than control values that persisted till the senile stage of the plant. Again after a fleeting increase of IAA-oxidase and RNase (Table 19) activities, a consistent decrease in the activities of the enzymes were recorded till senile stage of the plant. Atrinal-induced shortening of vine length and increase of stem circumference (Table 20) were however recorded throughout the observation periods.

Results of foliar application of the retardant at the sapling stage of 40-day-old plants thus indicate that higher levels of chlorophyll, protein, insoluble carbohydrate and RNA as well as enhanced activities of the anabolic enzymes like catalase and peroxidase in the retardant-treated plants maintained vital functional life of the plant for longer duration. Thus, after experiencing a transient set-back with respect to potential performance of the species at sapling stage, all the retardant-treated plants revealed higher metabolic status and showed enhanced plant potential throughout its life span. However, the initial inhibitory

effect may be justified by an immediate strong retardation action of Atrinal on plant metabolism which started relinquishing with the progress of plant age. The adverse effects thus did not at all persist for a longer duration. A perpetuating retardation action of the chemical at later stages of plant development was clearly reflected on the overall growth of the chayote plant as evident from the reduction of vine length and enhancement of radial growth of stem which persisted till the senile stage of plant growth.

Retardant-induced reduction in plant height is amply documented (Cathey, 1964; Lovett and Campbell, 1973; Guardia *et al.*, 1974; Clark and Fedak, 1977; Bhattacharjee, 1984; Bhattacharjee and Gupta, 1984) Cathey (1964) in his review lucidly reported the work of many workers on various physiological roles of growth retardants and the very common and significant visible effect of the chemicals is the shortening of plant height. Whitehead (1965) showed that both shortening and xeromorphism could be induced in sunflower through CCC application. Guardia *et al* (1974) observed that CCC and SADH efficiently reduced plant height and produced thicker as well as stronger stem in sunflower. Bhattacharjee (1984) reported that in sunflower shortening of plant height and increase of stem circumference were associated with profuse development mechanical tissues and enhanced lignification in stem. Effect of Atrinal on the alteration of plant growth and metabolism was studied by a number of workers (Bocion *et al.* 1975; De Silva *et al.* 1976; Hield *et al* 1978; Orson and Kofranek; 1978; Bhattacharjee and Gupta, 1984; Bhattacharjee *et al.* 1986, Mattia, 1984). Bocion *et al.* (1975) reported that Atrinal retarded the growth of a wide range of plant species which included cereals, cultivated as well as weed grasses and woody plants. Atrinal-induced inhibition of growth was observed by Shulmann and Lavee (1983) in grapevine and olive shoots. A number of reports exist in the literature that Atrinal like other conventional growth retardants, exert influence on plant metabolism (Bocion and De Silva, 1976; Gressel and Cohen, 1977; Zilkah and Gressel, 1978; 1979; 1980; Bhattacharjee and Gupta, 1981a,b and 1984). Inhibition of chlorophyll biosynthesis has been studied in *Zinnia*, *Chrysanthemum* and *Helianthus* (Arzee *et al.* 1977), Azalea (Bocion and De Silva, 1977a) *Helianthus annuus* (Purohit, 1979).

The existing literature pertaining to the retardant-induced effects on the changes in growth and metabolism thus, corroborate the overall findings of this investigation done with a different plant species.

In this study, Atrinal resulted in a significant increase of yield components like fruit weight as well as tuberous root weight per plant. The retardant also showed a tendency towards deferring plant senescence (Table 21). Increased crop yield as well as senescence delaying effect of Atrinal can be substantiated from the enhanced plant potential as evident from the biochemical analyses of this investigation. Unlike intact or defleshed sprouting whole fruit treatment, foliar treatment with the growth retardants at the sapling stage enhanced the levels of vital cellular components like chlorophyll, protein, carbohydrate, nucleic acids as well as the activities of the scavenger enzyme catalase and peroxidase particularly at the fruiting and senile stages of the plant. During active fruiting phase or assimilate filling phase of plants, developing fruits or grains function as reproductive sinks which show a strong sink demand and thus accumulate assimilates from the source leaves or contributory leaves (Bhattacharjee *et al.*, 1984; Milthrope and Moorby, 1988). Prolonged assimilate transport due to strong sink demand enhance plant capital, and delaying of the senescence of plants cause augmentation of yield in many plants (Bhattacharjee 1984; Bhattacharjee *et al.* 1986; Kumar and Purohit, 1997; Biswas and Ghosh 1999). In the present investigation, it seems apparent that increased crop yield in chayote is possibly due to maintenance of vital functional life of the source leaves by delaying of senescence which in turn efficiently transported assimilates for a longer duration.

Whatever might be the mechanism of senescence in this monocarpic vegetable crop, it seems quite likely that Atrinal-induced enhanced plant potential, deferred plant senescence, desired plant type modification and possibly prolonged assimilate transportation during fruit development cumulatively resulted in a substantial enhancement of yield. Some authors critically analysed scientific crop production as well as source-sink relationship in various crop plants including a few vegetable crops and came to the conclusion that a balanced source-sink relationship is an important determinant for crop yield (Thakur, 1975; Milthrope and Moorby, 1988; Biswas and Ghosh, 1999). Retardants, in

general, delay the onset of senescence in plants (Orchard and Lovett, 1976; Weaver, 1972; Bhattacharjee, 1984; Bhattacharjee et al; 1986). Retardant-induced delaying of seed senescence and consequent enhancement of seed potential in some species have been established (Bhattacharjee and Gupta, 1985; Bhattacharjee and Choudhuri, 1986; Chhetri et al., 1993; Basu, 1994; Rai et al. 1995; Bhattacharjee et al., 1999; <sup>^ Rai-2010</sup> Maity et al. 2000). Deferral of senescence in vegetables, cut flowers and even in mushroom and the resultant longevity have also been documented (Halevy, and Wittwer, 1966). While studying the processes of monocarpic senescence Nooden et al (1979) concluded that the prevention of the internally programmed degeneration might open a way to yield improvement. In the present study thus the augmented yield in the treated plants can be justified from the reported observations with respect to manipulation of source-sink, senescence as well as plant type.

Experiment on foliar treatment with growth retardants at preflowering stage and retardant followed by hormone treatment at the flowering stage seemed to be more interesting. In this experiment Atrinal, regardless of its concentrations, caused to increase the levels of chlorophyll and protein (Table 22) soluble and insoluble carbohydrates (Table 23), RNA and DNA (Table 24) as well as the activities of catalase and peroxidase (Table 25) enzymes. Such increases were further enhanced as a result of GA<sub>3</sub> and kinetin application at flowering stage (Table 29,30,31 and 32) . Again Atrinal-induced decrease in IAA-oxidase and RNase activities (Table 26) was decreased to a further extent in the GA<sub>3</sub>- and kinetin-treated samples (Table 33). Biochemical changes in leaves were associated with the changes in vine length and stem circumference (Table 27). While the retardant-induced reduction of vine length was substantially overcome in combined treatments with GA<sub>3</sub> and kinetin, stem circumference in the chemical treated plants was found to increase steadily throughout the observation periods in kinetin-treated plants (Table 34), and thus combined treatments were found more effective.

Unlike the results of foliar treatment of Atrinal at sapling stage, the consistent increase of chlorophyll, protein, soluble and insoluble carbohydrate, RNA and DNA levels as well as the activities of catalase and peroxidase enzymes indicate that their application at the preflowering stage (active log phase of growth) resulted in a steady and unflinching

enhancement of plant potential which persisted till senile stage. Further potentiation of the retardant-treated plants by GA<sub>3</sub> and kinetin, as evident from the biochemical changes and rejuvenated plant growth, is indicative of the fact the plants under combined treatment could successfully defer the inevitable internally programmed degeneration occurring during senescence. The data of this investigation thus prove the senescence deferral property of the chemicals.

In the physiology of plant senescence, it is now well established that senescence is accompanied by the decrease of chlorophyll and protein levels and/or increase of their degradation (Osbrone, 1967; Leopold and Kriedemann, 1975; Van Staden et al. 1988; Biswas and Ghosh, 1999). There are reports that the activities of enzymes like protease and IAA-oxidase increase and catalase activity decrease during senescence (Biswas, 1978; Bhattacharjee, 1984) and alteration of such senescence indices by growth retardants indicate their senescence deferral action. While studying the processes of monocarpic senescence in soybean Nooden et al (1979) concluded that prevention of the internally programmed degeneration might open a way for strengthening plant potential and consequent enhancement of yield. Whatever might be the mechanisms of senescence (Thomas and Stoddart, 1980; Thimann, 1980; Nooden and Leopold, 1988; Engvild, 1989; Nooden et al., 1997) it is now well documented that deferral of plant senescence results in an enhanced plant vigour and such invigouration in many occasions is associated with enhanced productivity (Biswas and Choudhuri 1978; Nooden et al. 1979; Bhattacharjee et al. 1984; Biswas and Ghosh 1999).

Results of yield analysis along with senescence of plants (Tables 28, 35) and flower productivity (Table 36) using growth retardants and growth promoters respectively at preflowering and flowering stages, as observed in the present investigation, seemed to be more encouraging. Strong senescence deferral action in conjunction with higher productivity particularly in combined treatments (Atrinal + GA<sub>3</sub> or Atrinal + Kinetin) can be explained by the enhanced potential of the plants by the growth retardant Atrinal and effective utilization of the potentiated vigour by stimulating the sink demand using GA<sub>3</sub> or kinetin at the appropriate stage i.e. flowering stage of plant development. Again, a significant increase of

flower numbers (Table 36). Particularly female flowers, in combined treatments (specially in Atrinal +GA<sub>3</sub>) showed an additive effect to the enhanced productivity. In fact, higher number of female flowers resulted in corresponding increase of fruit numbers and consequent increase of total yield of fruits per plant. Now the question arises why tuberous root weight and also weight of fruits per plant increased both in single (Atrinal only) and in combined treatments although the increase was distinctly differential in single and combined treatments. This observation can be well explained from the available literature on source-sink and translocation system relationship of plants and particularly that of monocarpic plants where a balance relationship of source, sink and translocation system plays a crucial role for optimum productivity.

In crop plants physiological basis of yield was demonstrated and it was shown that enhanced plant potential becomes futile if plants fail to exploit vigour by efficiently drawing assimilates to the reproductive sinks during fruiting (Evans, 1975; Bhattacharjee, 1984; Bhattacharjee et al. 1984; Milthrope and Moorby, 1988). In the present investigation at least two systems were vitalized i.e. source and sink, while translocation system remained unexplored. Potentiation of source system was well documented in this work where the treated plants got rejuvenated as evident even by visual appearance and this was biochemically substantiated by analysing some senescence variables like enhancement of chlorophyll, protein, carbohydrate, nucleic acids as well as activities of catalase and peroxidase enzymes and suppression of deleterious enzymes like IAA-oxidase and RNase during active fruit development and senile stages. Efficiency of the reproductive sinks was supposed to be enhanced by the hormonal (GA<sub>3</sub> and kientin) treatments during flowering stage where endogenous hormone-induced sink stimulation was further augmented by exogenous application of the hormones at the appropriate assimilate filling stage.

Hormone-directed translocation of assimilates is an well established phenomenon (Audus, 1959; Davies and wareing, 1965) Thomas, 1985). It is generally accepted that actively growing meristems and reproductive organs are the potential sinks for photosynthetically produced assimilates, and that activation or repression of apical sink and/or reproductive sinks may result in corresponding changes in growth and yield of plants

(Moorby, 1977). Growth retardants generally act through suppressing the apical sinks by reducing the hormonal levels therein and consequently by hindring the acropetal mobilization of assimilates (Cathey, 1964; Monselise and Luckwill, 1974; Hoad and Monselise, 1976; Ben-Gad et al., 1979). Bhattacharjee (1984) using  $^{32}\text{P}$  showed that feeding of  $^{32}\text{P}$  through contributory leaves of sunflower of some retardant treated plants resulted in a strong hindrance of  $^{32}\text{P}$  mobilization to the upper leaves and reproductive sinks of the capitulum. He further observed that concomitant with such hindered mobilization of  $^{32}\text{P}$  at the apical region basipetal mobilization was stimulated in the retardant treated plants.

Thus, from all the reported observations, it seems likely that in this investigation Atrinal induced positive factors of productivity like suppression of excess vigour and invigouration of plants but induced a negative factor for fruit production in chayote i.e. lowering of acropetal mobilization of assimilates, <sup>however this</sup> was overcome by exogenous application of  $\text{GA}_3$  and kinetin which possibly compensated or even enhanced sink demand. So, enhanced plant vigour in conjunction with activated reproductive sink resulted in a substantial increase of fruit yield. Again, enhanced yield of tuberous roots can be explained by the reported observation that during retardant-induced basipetal mobilization the tuberous roots acted as the alternate potential sinks at least till the growth promoters are applied at the flowering stage of the plant causing diversion of assimilates to the reproductive sinks. In the present investigation, thus Atrinal alone caused to enhance root yield, and combined treatments with Atrinal followed by growth promoters ( $\text{GA}_3$  and kinetin) caused to improve fruit yield. It has been demonstrated by many workes (Lorenzi *et al.* 1988, Ceccarelli and Lorenzi, 1990; Piaggese *et al.*, 1997) that in chayote plant hormones like IAA, cytokinin and  $\text{GA}_3$  appear during fruiting and developing fruits yield a considerable amount of the hormones. Thus enhancement of the levels of these hormoens by their exogenous application, as done in the present experiment, resulted in a remarkable enhancement of fruit yield.

Hormonal regulation of sex expression as well as flowering and fruit development in many plants including some cucurbits are well documented (Ghosh and Basu, 1982; 1983; 1984; Banerjee and Basu, 1991; 1992). Banerjee and Basu (1992) reported that  $\text{GA}_3$  and

ethrel enhance female flower production, stimulate fruit setting and fruit development in a monoecious cucurbit, *Momordica charantia*. Enhancement of both male and female flowers per plant by GA<sub>3</sub> application was demonstrated by Prakash (1977) in the same cucurbit. GA<sub>3</sub>, IAA and HMO, an oxidation product of IAA promoted female flowers in *Momordica* which resulted in yield improvement (Ghosh and Basu, 1983). Influence of Atrinal and IAA in increasing female flower production, decreasing the percentage of abortive female flowers and consequent augmentation of fruit yield was reported by Banerjee and Basu (1991).

In this observation with a different cucurbit (*Sechium edule*) masculinizing and feminizing effect of GA<sub>3</sub> and kinetin and corresponding enhancement of fruit yield is thus in agreement with reported results. In this investigation this floral stimulation property of the growth promoters was efficiently utilized after modifying plant growth and potentiating the chayote plants by prior application of the growth retardant Atrinal. Thus retardant and promoter-induced selective dual action on the plants caused significant augmentation of crop yield in this study.

Thus, it is concluded that selective concentrations of Atrinal might be used with a view to increasing crop yield of chayote plant, but selection of the optimum stage of the chemical application and exploitation of the imposed higher vigour through hormonal manipulation at a critical stage of plant growth are the important determinants for obtaining the most covetable result.