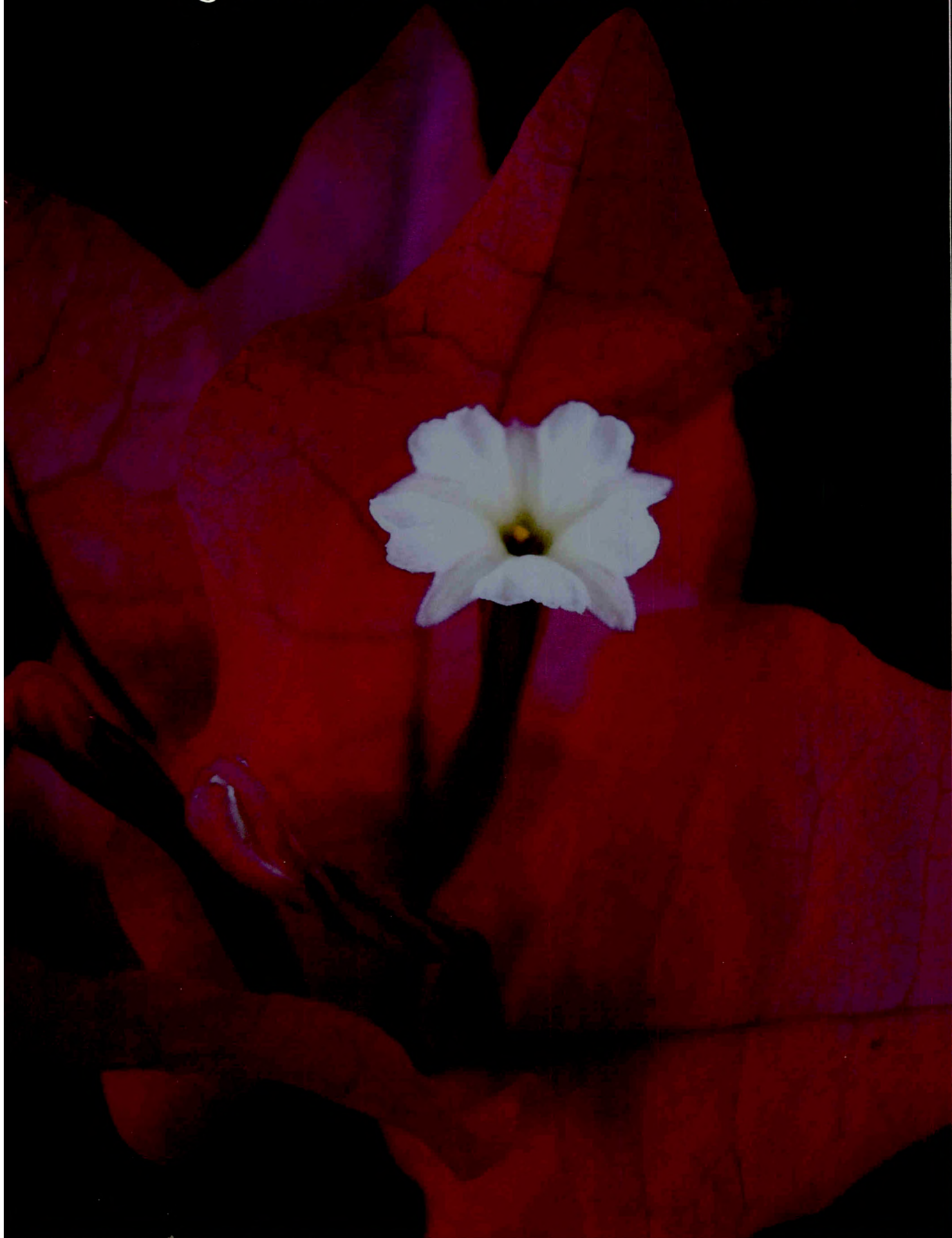


Results and Discussion



III. RESULTS AND DISCUSSION

A) Organic Constituents

1) Moisture content and dry matter analysis

Addicot and Lynch (1955) and Joshi and Mishra (1970) have mentioned that water must be readily available, if the cells of abscission zone are to function. The increase in moisture percentage may be due to more accumulation of ions in the senescent leaf so as to reduce the toxic effects of ions by dilution as indicated by Jennings (1968). Bole and Bharucha (1954) reported data on osmotic relationship in leaves of *A. alba* and concluded that older leaves always had greater water contents and higher osmotic pressure than younger ones. The more water in senescent leaves is helpful in the formation of abscission zone. In the present investigation we found an increase of moisture percentage in the senescent leaves of *Bougainvillea spectabilis*.

The results of moisture percentage depicted in table no. 1.

Total dry matter accumulation during leaf development is one aspect of leaf growth and it represents the assimilated carbon. All the accumulated dry matter of the leaf originates from photosynthesis. CO₂ uptake is on the input and dry matter accumulation on the output of the very complex system of photosynthesis where numerous processes are involved. But during such processes assimilated carbon which is either lost by respiration process or translocated out of the leaf. The main inputs of the system are carbon dioxide, water and solar energy. In the chloroplast, photosynthates are produced due

cascade of photosynthetic reactions. These photosynthates are necessary for growth and maintenance of the leaf or they are accumulated as leaf dry matter (Rane, 1987). Dry matter percentage is increased in senescent leaves as compared to young and mature leaves. In case of treated leaves with growth hormones GA, IAA and Kinetin the dry matter increased progressively. This observation is in accordance with the increased Ca^{++} in the senescent leaves of *B. spectabilis* as it is immobile. The results of dry matter percentage depicted in table no. 2.

Table no.1.

Effect of GA, IAA and Kinetin on moisture content in *Bougainvillea spectabilis* (Willd.)

	Control	GA	IAA	Kinetin
Pre-mature	73.47	76.27	70.10	68.42
Mature	66.67	66.15	67.39	70.49
Onset	73.08	64.53	66.37	69.01
Senescence	67.03	64.47	63.75	64.00

Values are expressed in % (Percentage).

Table no.2.

Effect of GA, IAA and Kinetin on dry matter content in *Bougainvillea spectabilis* (Willd.)

	Control	GA	IAA	Kinetin
Pre-mature	26.53	23.73	29.90	31.58
Mature	33.33	33.85	32.61	29.51
Onset	26.92	35.47	33.63	30.99
Senescence	32.97	35.53	36.25	36.00

Values are expressed in % (Percentage).

2) PHOTOSYNTHETIC PIGMENT STUDY

a. Chlorophylls

The effect of growth regulators on chlorophyll content has been studied. Influence of GA, IAA and Kinetin on the chlorophyll content at different stages of leaf development of *Bougainvillea spectabilis* is shown in the table and fig. 3.1 to 3.4. It is clear from the figure that GA is one of such growth hormone which shows decrease in chl a content during the mature stage to senescence stage.

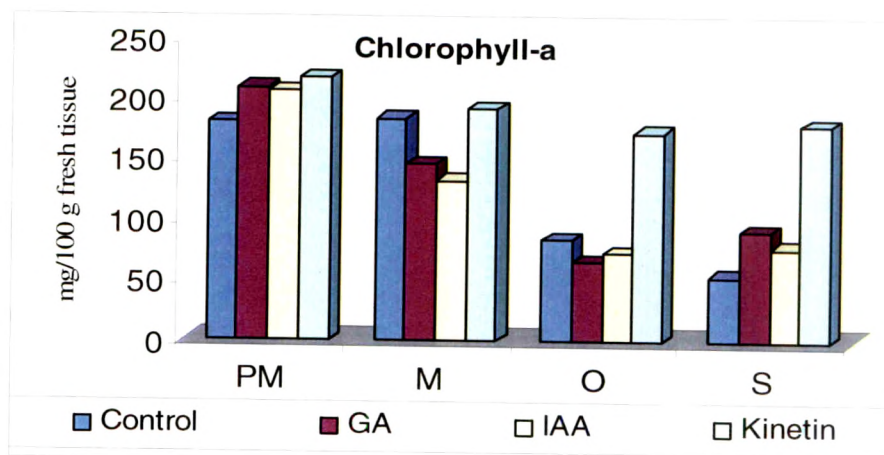
The effects of IAA treatment shows a similar pattern is observed in case of all the stages of leaf development as in GA treatment, however chlorophyll content is retained at high concentration over control due to the action of kinetin. Total chlorophylls decreased from premature to senescent stage due the influence of growth hormone GA. IAA treated leaves of *B. spectabilis* shows a similar pattern as that of the GA treatment. In case of kinetin treated leaves total chlorophylls are retained at much higher concentrations over to control during all the four stages of leaf development.

Chlorophyll a/b ratio is decreased in all treatments at premature stage to senescent stage. It is parallel with the control. However Kinetin treated plants shows an increase in chl a/b ratio at onset of senescence stage and senescent stage.

Table and Fig. 3.1.

Effect of GA, IAA and Kinetin on chlorophyll a in *Bougainvillea spectabilis* leaves

	Control	GA	IAA	Kinetin
PM	180.7	209.2	206.0	217.5
M	183.3	146.0	132.3	191.1
O	84.3	65.5	72.9	172.3
S	53.8	90.7	77.4	178.5

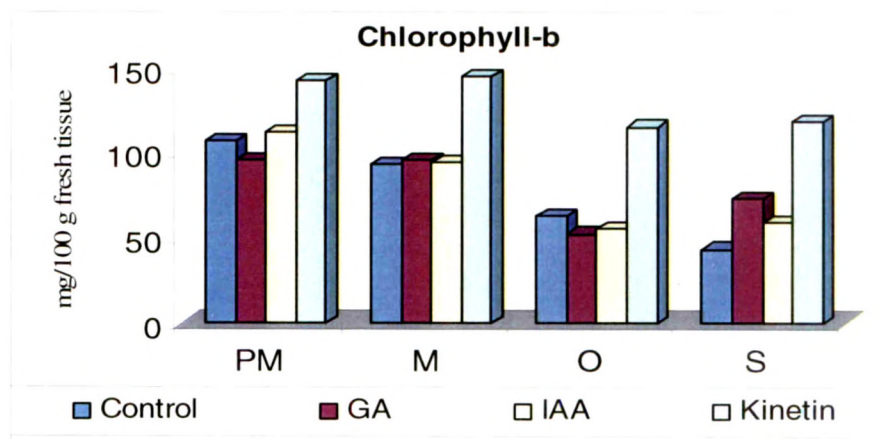


PM: Premature, **M:** Mature, **O:** Onset of senescence and **S:** Senescence

Table and Fig. 3.2.

Effect of GA, IAA and Kinetin on chlorophyll b in *Bougainvillea spectabilis* leaves

	Control	GA	IAA	Kinetin
PM	107.5	95.7	112.2	142.4
M	93.2	95.8	94.7	145.3
O	62.9	52.3	55.4	115.4
S	43.4	73.2	59.7	118.3



PM: Premature, M: Mature, O: Onset of senescence and S: Senescence

The effect of kinetin treatment only showed an increase in chl a/b ratio because of retention of chlorophylls and greening (rejuvenation) as a effect of kinetin. This is delaying effect on senescence.

Pretreatment with ABA inhibits the increase in gibberellin levels in response to red light, but the inhibitory effect of ABA or unrolling cannot be ascribed only to its effect on GA levels. Pretreatment with red light reduces lag-phase in chlorophyll development. Wheat leaf segments are subsequently exposed to white light: the effect of red light may be replayed by pretreatment with kinetin, but gibberellic acid is relatively ineffective in this respect (Beevers *et al.*, 1970).

According to Reiss and Beale (1995), external calcium requirement for light induction of chlorophyll accumulation and its enhancement by red light and cytokinin pretreatment in excised etiolated cucumber cotyledons. They showed the enhancement of chlorophyll accumulation by cytokinin.

In *Nicotiana glutinosa* leaves loss of proteins and chlorophyll was advanced by the action of ABA (190M). While in contrast IAA (10M) and GA₃ (50M) did not alter the normal patterns of change, whilst Kinetin (50M) delayed the loss of protein and chlorophyll.

The senescence delaying regulator gibberellin-A₃ (GA₃) inhibited the effect of ethylene on chlorophyllase transcript accumulation (Jacob-Wilk *et al.*, 1999). In a hormonal supplemented medium *Pinus pinaster* cotyledons showed

an increase in the polar lipid content and chlorophyll content and which appeared to strongly correlated with supplement (Tranvan *et al.*, 1988).

The increase in chlorophyll levels was proportional to cytokinin concentration and was apparent at concentration as low as 0.001 mg/liter. Gibberellic Acid, Indole Acetic Acid did not cause a similar increase in chlorophyll levels. This effect of cytokinin on chlorophyll formation is valuable as a simple, rapid bioassay for cytokinin (Fletcher and Mc Cullagh, 1971)

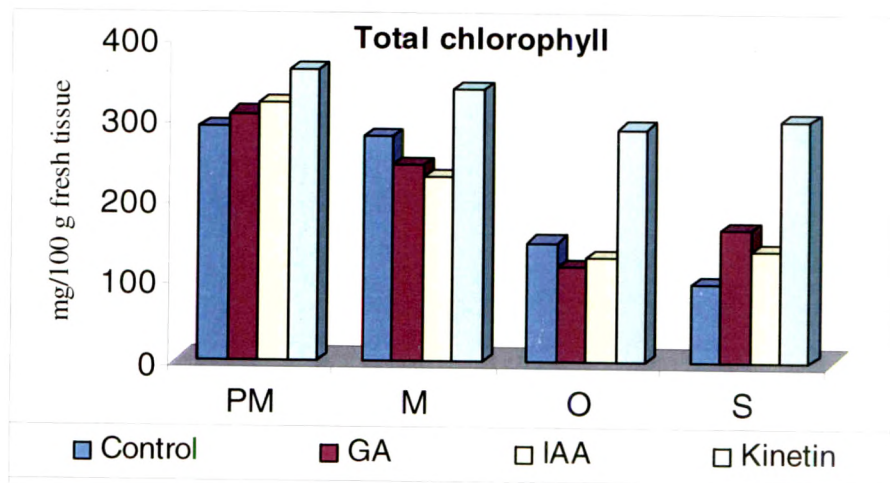
The higher chlorophyll a:b ratio generally signifies a higher photosystem – I activity i.e. cyclic photophosphorylation (Black and Mayne, 1970). The lowest chlorophyll a:b ratio due to a raising of the level of Chl:b and this indicates that both photosystems I and II might be active and highest a:b ratio indicative of higher cyclic photophosphorylation, a tendency towards an type of photosynthesis (Chen *et al.*, 1969). Dyer and Osborne (1971) reported that leaves of plant approaching senescence, the synthesis of nucleic acids and protein becomes progressively less and total levels fall. Quantitative changes in the pigment composition are also found in senescing leaves.

Choe and Thimam (1975) who observed rapid loss of both chlorophylls and carotenoids during aging of isolated chloroplast in light where as dark incubation of chloroplasts significantly check this loss. According to Ashrif *et al.*, (1994), the role of chlorophylls in photosynthesis is well established but

Table and Fig. 3.3.

Effect of GA, IAA and Kinetin on total chlorophyll in *Bougainvillea spectabilis* leaves

	Control	GA	IAA	Kinetin
PM	288.1	304.9	318.1	359.8
M	276.5	241.8	227.0	336.4
O	147.2	117.8	128.4	287.7
S	97.2	163.9	137.1	296.8

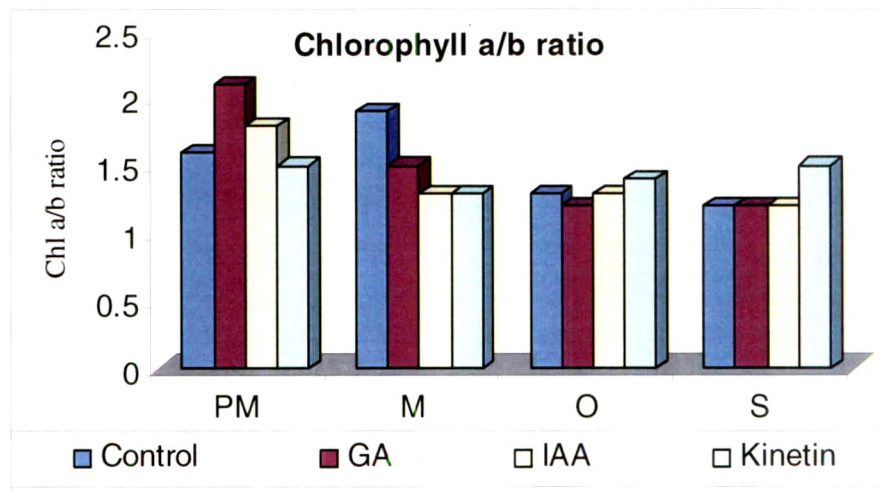


PM: Premature, **M:** Mature, **O:** Onset of senescence and **S:** Senescence

Table and Fig. 3.4.

Effect of GA, IAA and Kinetin on chlorophyll a/b ratio in *Bougainvillea spectabilis* leaves

	Control	GA	IAA	Kinetin
PM	1.6	2.1	1.8	1.5
M	1.9	1.5	1.3	1.3
O	1.3	1.2	1.3	1.4
S	1.2	1.2	1.2	1.5



PM: Premature, M: Mature, O: Onset of senescence and S: Senescence

the relationship between chlorophyll content and the rate of photosynthesis is equivalent

According to Salunkhe (1997), chlorophylls are declined in the senescent leaves of both the sub species of *Ipomoea carnea* however the treated leaves with growth hormones like GA, IAA and Kinetin show retention of in chlorophylls resulting in delaying of senescence.

b. Carotenoids

The carotenoids play a secondary role during the process of photosynthesis and general trend of carotenoids pigments during leaf senescence is similar to that of chlorophylls (Sestak, 1985). Although there are many studies of carotenoids accumulation during development of attached leaves. A very few attempts have been made to study the fate of carotenoids during induced senescence. Thimann *et al.*, (1977) showed that etiolated oat leaves allowed to senescence in dark; loose their carotenoids at about the same rate as green leaves of the same age loose their chlorophylls.

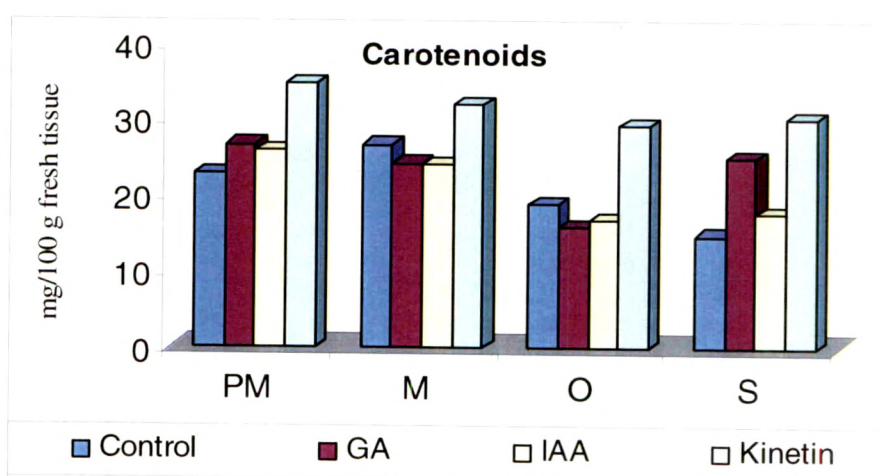
Panigrahi and Biswal (1979) found that chlorophylls and carotenoids are degraded but the rate of degradation of carotenoids is faster than that of chlorophylls. However in case of detached barley leaves Biswal and Mohanty (1976) found that chlorophylls are degraded much faster than carotenoids.

Rane (1991) noticed the carotenoids loss in groundnut leaves is more pronounced under continuous darkness than under natural light. It is also observed that carotenoids decreased during leaf senescence are comparatively significant in groundnut leaves during induced leaf senescence. Salunkhe (1997) showed the accumulation of carotenoids in senescent leaves of *Ipomoea carnea*. He also found that the treatment of GA and IAA accelerated the breakdown of carotenoids as compared to control. However the kinetin retarding the breakdown rate of carotenoids and delaying the senescence.

Table and Fig. 3.5.

Effect of GA, IAA and Kinetin on carotenoids in *Bougainvillea spectabilis* leaves

	Control	GA	IAA	Kinetin
PM	22.88	26.72	26.04	34.80
M	26.76	24.32	24.20	32.08
O	19.04	15.96	16.72	29.40
S	14.80	25.12	17.80	30.20



PM: Premature, M: Mature, O: Onset of senescence and S: Senescence

Effect of GA, IAA and Kinetin growth regulators on the carotenoids level at different leaf stages of *B. spectabilis* is depicted in table and fig.3.5. It is evident from the figure that above treatment has its impact at the premature stage and senescent stage by increased carotenoids level over to control.

An increase in the level of carotenoids is observed at both premature and senescent stage, while it is decreased in rest of the stages of leaf development. The clear-cut influence of kinetin treatment is observed in all the four stages of leaf development in *B. spectabilis* by an increased and retention of carotenoid content.

Studies on seedlings lacking carotenoids showed that photo-oxidation inhibited the transcription of a set of nuclear genes (Mayfield and Taylor 1984, 1987, Oelmuller and Mohr 1986, Oelmuller et al. 1986). Because carotenoids are the precursors of ABA (reviewed by Taylor 1991, Zeevaart et al. 1991),

3) Total polyphenols

Consequence of GA, IAA and Kinetin on the content of polyphenols at different leaf development stages of *B. spectabilis* is shown in table and fig.3.6. It is clear from the figure that Kinetin treated plant leaves shown an increase in polyphenol content at mature and senescent stage.

Polyphenols are the large and diverse class of compounds, many of which are occur naturally. Most of these phenolics are intermediates and derivates of the shikimate and phenylpropanoid pathways (Cheng and Breen, 1991).

A range of plant polyphenols are either being actively developed or currently sold as a dietary supplements and / or herbal medicines. Although they play a key role in nutrition (non-nutrients), many of them have properties including antioxidant, antimutagenic, antioestrogenic, anticardiogenic and anti-inflammatory effects, that may preventing diseases and protecting stability of genome (Ferguson, 2000).

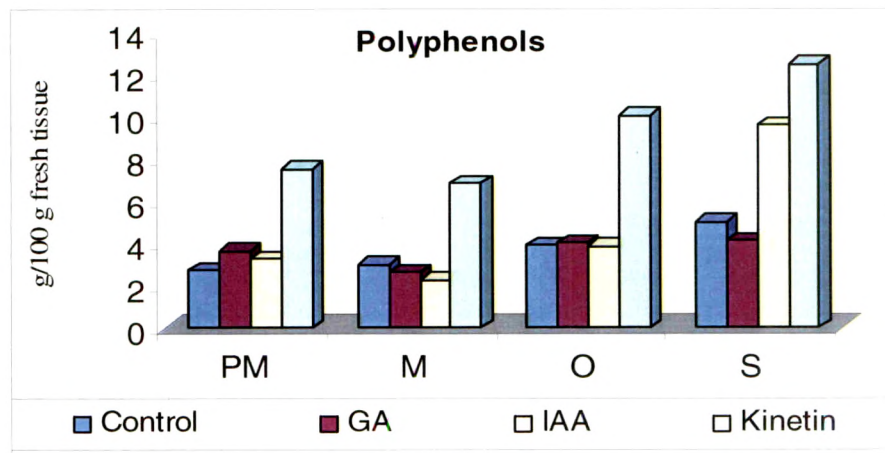
The early stage of development of enzyme phenyl alanine ammonialyase (PAL) is less sensitive to high hormonal levels than in polyphenol synthesis. High auxin levels may also inhibit some other compounds of the PAL biosynthetic pathway (Davies, 1972).

Natural leaf senescence proceeds through an orderly programme of events referred to as the 'senescent syndrome'. Leaf senescence consists of primarily, but not exclusively a set of degradative and remobilization activities

Table and Fig. 3.6.

Effect of GA, IAA and Kinetin on total polyphenols in *Bougainvillea spectabilis* leaves

	Control	GA	IAA	Kinetin
PM	2.75	3.64	3.28	7.53
M	3.00	2.60	2.25	6.85
O	3.92	3.96	3.82	10.07
S	5.00	4.07	9.60	12.50



PM: Premature, **M:** Mature, **O:** Onset of senescence and **S:** Senescence

that salvage valuable nutrients by reallocation to the seed or other viable parts of the plant (Chandlee, 2001).

4) Carbohydrate status

Carbohydrates are the major micronutrients containing carbon, hydrogen and oxygen atoms in their structures. The carbohydrates are divided roughly into three groups the monosaccharides, the oligosaccharides and the polysaccharides. Carbohydrates are the potential source of energy, their degradation produces the energy utilized in many of the synthetic reactions of the cells. Sucrose is the principal form of carbohydrates which are transported in higher plants. The capacity of the translocation system for the export does not seem to become impaired until the leaf has reached a very advanced stage of senescence (Milthorpe and Moorby, 1969). The decline of photosynthesis and carbohydrates is associated with rapid translocation of hydrolysis product out of the senescing leaf (Lewington and Simon, 1969). Reducing sugar and total sugar decreased during senescence. The senescent leaves attached to the plant do not show accumulation of carbohydrates (Jamale and Joshi, 1976). Thimann *et al.*, (1974), stated that intact leaves of the plant show migration of sugars towards roots.

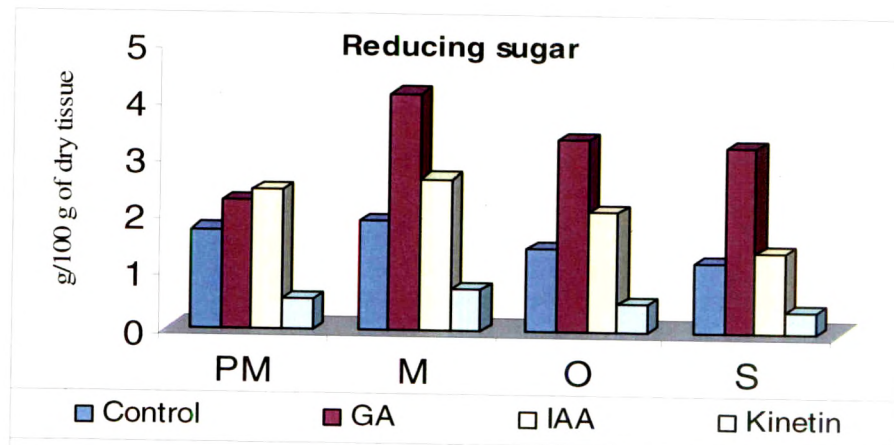
a. Reducing sugars (RS)

Influence of GA, IAA and Kinetin on the carbohydrate content at different leaf stages of *B. spectabilis* is shown in table and fig. no. 3.7 to 3.9. It is clear from the table and figure no.3.7, that the level of reducing sugar is increased in all four leaf stages due to the application of GA and IAA. But the

Table and Fig. 3.7.

Effect of GA, IAA and Kinetin on reducing sugars in *Bougainvillea spectabilis* leaves

	Control	GA	IAA	Kinetin
PM	1.74	2.24	2.45	0.53
M	1.90	4.14	2.65	0.71
O	1.44	3.36	2.09	0.50
S	1.21	3.25	1.40	0.39

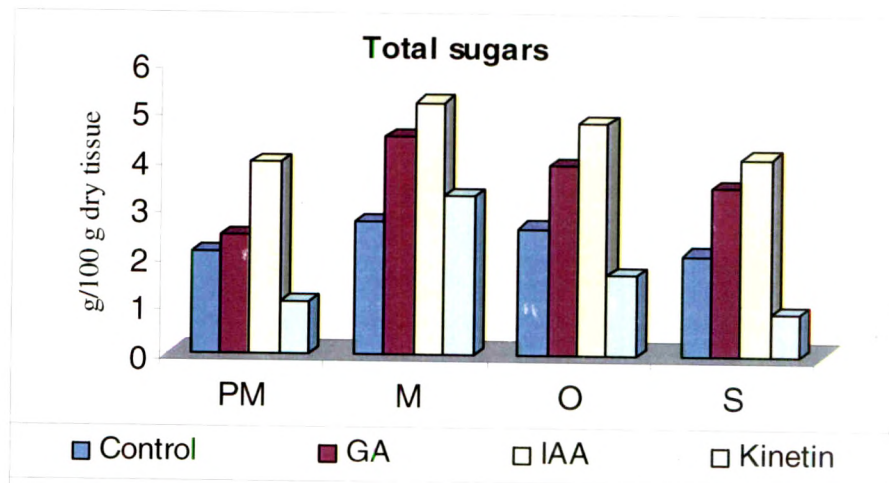


PM: Premature, M: Mature, O: Onset of senescence and S: Senescence

Table and Fig. 3.8.

Effect of GA, IAA and Kinetin on total sugars in *Bougainvillea spectabilis* leaves

	Control	GA	IAA	Kinetin
PM	2.10	2.45	3.95	1.08
M	2.72	4.50	5.20	3.25
O	2.61	3.91	4.80	1.67
S	2.07	3.48	4.07	0.89

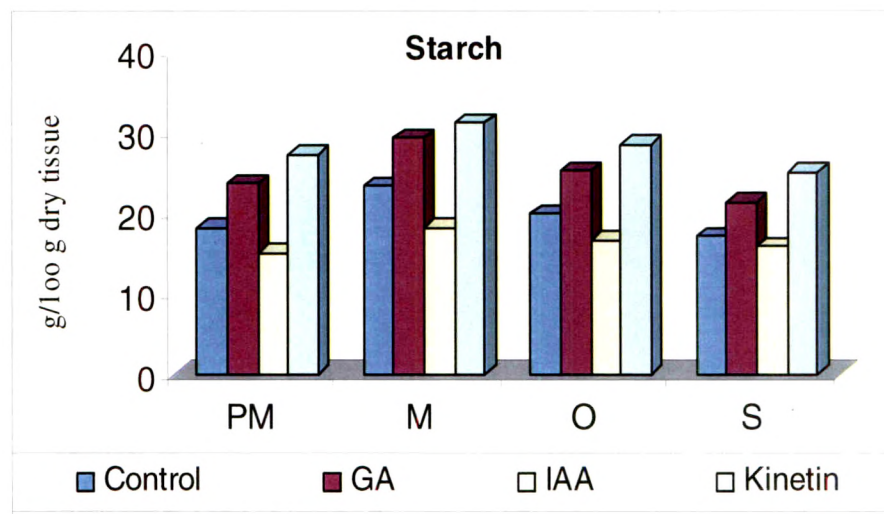


PM: Premature, **M:** Mature, **O:** Onset of senescence and **S:** Senescence

Table and Fig. 3.9.

Effect of GA, IAA and Kinetin on starch in *Bougainvillea spectabilis* leaves

	Control	GA	IAA	Kinetin
PM	18.14	23.64	14.99	27.18
M	23.19	29.34	18.09	31.15
O	19.87	25.08	16.57	28.44
S	16.99	21.29	15.82	25.03



PM: Premature, M: Mature, O: Onset of senescence and S: Senescence

decrease in the content of the reducing sugar is observed by the action of kinetin.

b. Total sugars (TS)

The level of total sugar (Table and fig. 3.8) is also increased in all four leaf stages due the application of GA and IAA. The application of kinetin has reduced the level of total sugars all leaf stages except mature leaf stage.

In case of total sugars a similar kind of pattern as that of the reducing sugar i.e., an increase under the influence of GA and IAA and decreased in kinetin treatment.

c. Starch

Starch is polysaccharides containing homo-dimers of amylose and amylopectin. Much of the sugars produced in the photosynthesis are converted to starch, which is deposited in the plant tissue as starch grain (Rane, 1987). The results of starch content are depicted in table and fig. 3.9. It is clear from the figure that starch is elevated in all four leaf stages due to the application of GA and kinetin, while an opposite trend is noticed in case of IAA treatment.

In case of senescent stage of plant leaves an elevation of starch is noticed due to the action of GA and kinetin, while a slight decrease is noticed due the action of IAA.

B) Inorganic Constituents

1) Sodium (Na⁺)

Sodium is an important macronutrient which controls the plant growth and development (Joshi and Mishra, 1970). They observed higher content of Na in senescent leaves. According to them accumulation of Na⁺ and Cl⁻ in the senescent leaves might have affected the anabolic processes particularly photosynthesis. Hyder (1971) noted similar pattern of Na⁺ accumulation in *Citrus* leaves during the various leaf growth stages. He observed that K⁺ concentration was higher than Na⁺ in young leaf but towards maturity there was inverse amount between Na⁺ and K⁺. (Epistein and Hegman 1952; Brownwell and Crossland, 1972) indicated that C4 plants need more Na⁺ than C3.

Accumulation of Na along with chloride may be toxic to leaf metabolism (Joshi and Mishra, 1970). Ambike and Karmarkar (1975) have reported significant increase in Na⁺ content during senescence of *Kalanchoe pinnata*. They suggested that the high amount of Na⁺ accumulation accounts for the corresponding decrease in organic acid. Bhivare (1984) showed that

Table.4.

**Effect of GA, IAA and Kinetin on Na content in
Bougainvillea spectabilis (Willd.)**

	Control	GA	IAA	Kinetin
Pre-mature	0.265	0.112	0.150	0.150
Mature	0.130	0.140	0.140	0.130
Onset of senescence	0.112	0.138	0.130	0.130
Senescence	0.202	0.130	0.112	0.132

Values are expressed in g / 100 g dry tissue

higher content of Na in the senescent leaves of the plant are correlated with increased hydration, decreased K content and disturbed photosynthetic carbon metabolism. Upadhey (1986) reported significant accumulation of mono-valent cation in the senescing and senescent leaves and Na content is increased dramatically during senescence. According^{to} Naidu and Swamy (1996), the leaf Na concentration increases slightly throughout the life span of leaves until senescence. Our results also show Na accumulation in senescent leaves of *B. spectabilis* (Table no. 4). In the present investigation the effect of growth hormones on Na also studied and the result indicates no accumulation of Na in senescent leaves which may be due to mobilization of Na.

2) Potassium (K⁺)

Potassium (K) helps plants to overcome drought stress; improves winter hardiness; increased disease resistance; improves the rigidity of stalks; leaches from soil; mobile in plant. Deficiency of K shows reduced growth; shortened internodes; margins of older leaves become chlorotic and burn; necrotic (dead) spots on older leaves; reduction of lateral breaks and tendency to wilt readily; poorly developed root systems; weak stalks. K may affect the uptake of other positive ions such as Mg and Ca.

According to Soni *et al.*, (1970) K is indispensable for plant growth and it is withdrawn from the senescent leaves. Suelter, (1970) has listed not less than 58 enzymes which require mono-valent cation for their maximal activity. Potassium is also involved in the mechanism of ATP generation. It is also

Table.5.

Effect of GA, IAA and Kinetin on K content in *Bougainvillea spectabilis* (Willd.)

	Control	GA	IAA	Kinetin
Pre-mature	1.68	1.70	1.30	1.60
Mature	1.50	1.70	1.20	2.00
Onset of senescence	1.42	1.30	1.70	1.90
Senescence	1.40	1.00	1.30	1.60

Values are expressed in g / 100 g dry tissue.

major osmotically active component in plant cells contributing to cell turgor and enhancing the capacity of the plant cell to retain water.

Potassium is highly mobile in the phloem. Its utilization is therefore efficient in the sense that it is readily redistributed from older leaves to young growing organ. Effect of potassium deficiency on CO₂ fixation and translocation in groundnut was studied by Basha and Rao (1981). They further noticed that K⁺ deficiency had direct negative effect on translocation. Jamale and Joshi (1978), reported that K⁺ level falls down during senescence in mangrove. A decline in K⁺ status of senescent leaves has been noticed by Deshpande and Nimbalkar (1981) in *Cajanus cajan*. This trend was noticeable in experiments of Waughman and Ballmay (1981) with 21 plant species. Bengtsson and Jenson (1983), studied uptake and distribution K⁺ in Cucumber leaves of different age and noticed that K⁺ concentration decreased with increasing leaf age. Salunkhe (1997) reported that decrease of K content in the senescent leaves of *Ipomoea carnea*.

Our results show that there is considerable decrease in potassium content in senescent leaves of *B. spectabilis*. However the effect of GA, IAA and kinetin on K⁺ content is studied (Table no.5). GA and IAA treatment shows good mobilization while the kinetin maintains higher level of K⁺ in senescent leaves. Potassium and nitrogen are highly phloem mobile elements, and their re-utilization leads to rapid decline in their level in vegetative parts, thereby

inducing earlier senescence (Marschner, 1995). Our results show similar trend in the senescent leaves of *B. spectabilis*.

3) Calcium (Ca⁺⁺)

Calcium (Ca) absorbed as Ca⁺⁺; it is moderately leachable; it has limited mobility in plant; essential for growth of shoot and root tips; reduces the toxicity of aluminum and manganese. Deficiency of Ca results in inhibition of bud growth; roots can turn black and rot; young leaves are scalloped and abnormally green; leaf tips may stick together; cupping of maturing leaves; blossom end rot of many fruits, pits on root vegetables; stem structure is weak; premature shedding of fruit and buds. Ca interferes with Mg absorption; high Ca usually causes high pH which then precipitates many of the micronutrient so they become unavailable to the plant. Ca is rarely deficient if the correct pH is maintained; too much or too little water, can affect Ca relationships within the plant causing deficiency in the location where Ca was needed at the time of stress

Calcium is the most important essential element in the plant nutrition. The calcium functions as metallic enzyme activator, membrane stabilizer and in cell wall structure. Calcium is chief constituent of the middle lamella and in this region it is in the form of Ca-pectate. An important function of calcium is to stabilize membrane and selective ion uptake. Calcium controls the Na⁺ and K⁺ transport in plants.

High saline environments cause cytosolic accumulation of calcium which is a potent signal for stress responses that can result in either adaptation or death (Hasegawa et al., 2000). Young growing shoots are big competitors for calcium. So all adequate measurements for growth control are favourable for calcium content in the fruit (Bloksma and Jansonius, 2000).

Table.6.

**Effect of GA, IAA and Kinetin on Ca content in
Bougainvillea spectabilis (Willd.)**

	Control	GA	IAA	Kinetin
Pre-mature	5.31	3.60	3.00	2.20
Mature	4.20	2.80	4.20	2.60
Onset of senescence	3.27	2.60	3.00	3.00
Senescence	3.75	3.40	4.00	3.40

Values are expressed in g / 100 g dry tissue.

2% Ca induces a better preservation of membrane integrity during the storage period and delaying the senescence process (Barreiro *et al.*, 2003). Molisch (1938) points out that calcium oxalate and carbonate are precipitated above the cell walls of plants and increase with age. He suggests the possibility that calcium increases with age in the cell membrane but offers no evidence to support this view. Our result depicted in table no.6 show more Ca in younger leaves (Premature) as it required as metabolic enzyme activator, membrane stabilizer, cell wall structure and constituent of middle lamellae. Ca accumulation is also recorded in senescent leaves this is due less mobile nature. Growth hormone less effect to alter the Ca pattern in *B. spectabilis*.

4) Magnesium (Mg)

Magnesium absorbed as Mg^{++} ; leaches from sandy soil; mobile in plant Mg deficiency cause reduction in growth; yellowish, bronze, or reddish colour of older leaves, while veins remains green; leaf margins may curl downward or upward with a puckering effect. Mg interferes with Ca uptake; small necrotic spots in older leaves; smaller veins in older leaves may turn brown; in advanced stage, young leaves may be spotted. Mg is commonly deficient in foliage plants because it is leached and not replaced; Mg can be absorbed by leaves if sprayed in a weak solution; dolomitic limestone can be applied in outdoor situations to rectify a deficiency. Magnesium imparts

Table.7.

**Effect of GA, IAA and Kinetin on Mg content in
Bougainvillea spectabilis (Willd.)**

	Control	GA	IAA	Kinetin
Pre-mature	2.351	1.827	3.532	1.705
Mature	1.218	1.827	1.096	1.340
Onset of senescence	1.119	1.096	1.705	1.461
Senescence	1.024	1.235	1.827	1.761

Values are expressed in g / 100 g dry tissue.

the most important constituent of chlorophyll molecule therefore essentially required for the synthesis of chlorophylls and metallic co-factor of many enzymes. Magnesium also essentially functions as a bridging element for the aggregation of the ribosomal subunits. Mg is also the component of RNA-polymerases; it is required for synthesis of RNA in the nucleus.

Generation of ATP (ADP + ip) has an absolute requirement of Mg^{++} as a bridging component between ADP and enzyme. Another key reaction of Mg^{++} is modulation of RuBP carboxylase in the stroma of chloroplast (Sugiyama *et al.*, 1968). High Mg is required for ribulose 1, 6 diphosphatase regulation, assimilation and partitioning between starch synthesis and triose phosphate export in the chloroplast. Mg required for enzymes of ammonia assimilation like glutamine and glutamate synthetase (O'Neal and Joy, 1974).

The Magnesium content is depicted in Table.7. It is clear from the table that Magnesium content declined with increasing leaf age of *Bougainvillea spectabilis*. Similar pattern is seen in GA, and IAA treated leaves; however kinetin treated leaves showed an increased Magnesium content. This is because of retention of chlorophylls and rejuvenation as a effect of kinetin.

5) Iron (Fe)

Iron absorbed as Fe^{++} , Fe^{+++} ; accumulates in the oldest leaves and is relatively immobile in the phloem; necessary for the maintenance of chlorophyll. Fe deficiency leads to interveinal chlorosis primarily on young

Table.8.

**Effect of GA, IAA and Kinetin on Fe content in
Bougainvillea spectabilis (Willd.)**

	Control	GA	IAA	Kinetin
Pre-mature	0.0184	0.0211	0.0388	0.025
Mature	0.0176	0.0398	0.0186	0.0226
Onset of senescence	0.0237	0.0267	0.0246	0.0204
Senescence	0.0211	0.0355	0.0177	0.0270

Values are expressed in g / 100 g dry tissue.

tissue, which may become white; foliar fertilization will temporarily correct the deficiency; may be deficient in centipede grass where pH and P are high.

The Iron content is depicted in Table. 8. It is clear from the table that iron content is increased in the treated leaves of *Bougainvillea spectabilis*. Growth hormones stimulates the metabolism and due to which Fe content increased. Kinetin treated leaves retain fairly good amount of Fe in all the stages of leaf senescence of *Bougainvillea spectabilis*. Iron uptake, transport, and storage is tightly regulated to prevent both iron deficiency and toxicity, thus ensuring optimal plant development. Various transporters are required to achieve these iron fluxes (Curie and Briat 2003). Plant iron storage takes place in the apoplasmic space, in the vacuoles (Briat and Lobreaux 1998),

7) Phosphorus (P)

Phosphorus (P) promotes root formation and growth; affects quality of seed, fruit and flower production; increased disease resistance; does not leach from soil readily; mobile in plant, moving to new growth. Deficiency of Phosphorus leads to reduced growth; leaves dark green; purple or red color in older leaves, especially on the underside of the leaf along the veins; leaf shape may be distorted; thin stems; limited root growth. High P interferes with micronutrient and N absorption; used in relatively small amounts when compared to N and K; availability is the lowest in cold soils.

Table.9.

Effect of GA, IAA and Kinetin on P content in *Bougainvillea spectabilis* (Willd.)

	Control	GA	IAA	Kinetin
Pre-mature	3.49	2.50	0.92	0.31
Mature	1.50	1.60	1.40	1.34
Onset of senescence	1.78	2.16	0.76	1.08
Senescence	1.93	1.16	1.16	0.33

Values are expressed in mg / 100 g dry tissue.

The phosphorus content is depicted in Table.9. It is clear from the table that phosphorus content showed linear decrease with age of *Bougainvillea spectabilis*. But at the last phase of leaf development it is increased considerably. GA and IAA treated leaves maintain enough phosphorus content, which also shows retention at senescent stage of both these treatments.

The decrease in P concentration in the leaves might be due to plant translocation of P to fruits; (Aldana, 2005). Senescence of older leaves is triggered by a deficiency in a mobile element such as P; young leaves remain healthy, presumably because of nutrient remobilization from the older leaves to reproductive structures and younger leaves needed for photosynthesis (Smart, 1994). Leaf senescence is a common response to inadequate nutrient supply. When leaf senescence takes place, RNA, protein, and other N- and P-containing compounds are broken down to inorganic and soluble organic forms. Up to 90% of the maximum leaf N and P, 70% of the K, but none of the Ca is translocated out of senescing leaves before abscission (Brady, 1973; Williams, 1948 and 1955). In cereals, root absorption capacity declines with age, and mobilization of nutrients from older senesced leaves provides the bulk nutrients in the grains (Langer, 1966; Pitman and Cram, 1977; Williams, 1948 and 1955). Therefore, as plant nutrient status and the quantity of mobile nutrients in leaves decline, reproductive output declines (Chapin, 1980). Organic forms of phosphorus which are much less accessible for plant uptake (Lambers et al., 1998). Phosphorus plays a very important role in tree

physiology. In addition to its function in energy storage and in non-cyclic electron transport, phosphorus supply is related to plant Rubisco concentration (Warren and Adams,2002). Phosphorus content, declines with age by as much as 40 to 60 % (Walker & McComb 1988).

C) BIOCHEMICAL STUDIES

1) Enzyme studies

a. Enzyme Nitrate Reductase (NR) (EC 1. 6. 6. 1)

Effect of GA, IAA and Kinetin on the activity of enzyme nitrate reductase at four different leaf stages of *B. spectabilis* is depicted in table and fig. no. 10.1. It is clear from the figure that the activity of enzyme nitrate reductase is stimulated due the application of all the three growth regulators in all four leaf stages as compared to control.

A consequence of the growth regulators GA and Kinetin showed an enhanced activity of enzyme nitrate reductase during all four stages of leaf development. A similar kind of trend is noticed with IAA but at the senescent stage decrease in activity is recorded.

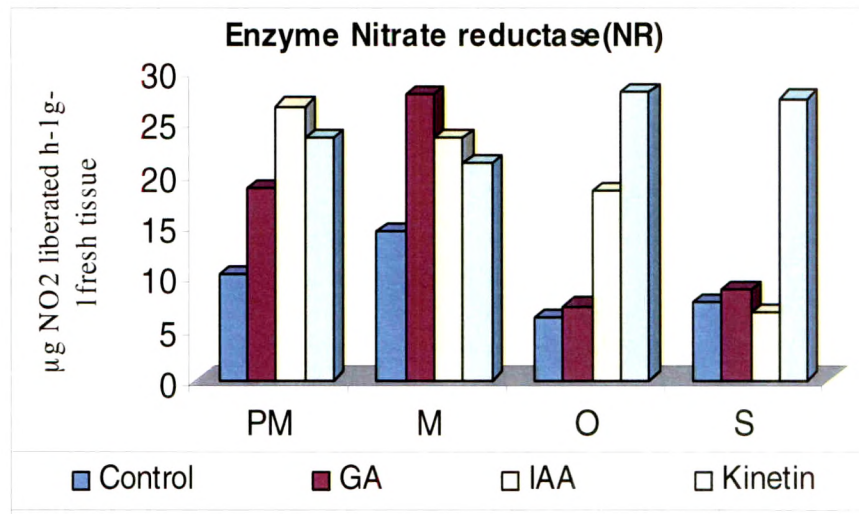
In the last but vital phase of leaf development i.e., senescent stage during which the translocation of various mineral and content is advanced due to the age factor. At this phase a decrease is seen in the activity of NR, due to action of IAA but an increased activity shown by the plant leaves by the consequence with GA and Kinetin.

Enzyme NR catalyzes the first step; the enzyme system consists of reduced pyridine nucleotide (NADPH or NADH) as an electron donar, Flavin-adenine dinucleotide (FAD) and molybdenum. According to Guereo *et al.*, (1981) during nitrate reduction electrons are directly transferred from

Table and Fig. 10.1.

Effect of GA, IAA and Kinetin on enzyme nitrate reductase in *Bougainvillea spectabilis* leaves

	Control	GA	IAA	Kinetin
PM	10.20	18.50	26.56	23.45
M	14.39	27.60	23.51	21.08
O	6.03	7.11	18.41	27.81
S	7.50	8.69	6.53	27.29



PM: Premature, **M:** Mature, **O:** Onset of senescence and **S:** Senescence

molybdenum to nitrite. The activity of this enzyme is a rate limiting step in nitrogen assimilation as this enzyme determines overall assimilation of nitrate. According to Austin *et al.*, (1978) there is a positive correlation between leaf nitrate reductase and growth rate.

Saxena and Saxena (2002) studied nitrate reductase activity and chlorophyll content in Sphagnum as affected by kinetin, while kinetin application enhanced the chlorophyll and nitrate reductase activity. Saroop *et al.*,(1999) studied the role of phytohormones in nitrate uptake and *in vivo* nitrate reductase activity of mustard cotyledons. *In vivo* nitrate reductase activity and nitrate uptake in gibberellic acid (GA)-and kinetin (KN)-treated mustard (*Brassica juncea* Coss. cv. T-59 Varuna) seedlings grown in the presence of light were investigated. They showed that Kinetin inhibited uptake of NO_3 and promoted *in vivo* NR activity, while although GA promoted uptake, *in vivo* NR activity was almost equal to distilled water (DW) control.

It is therefore understood that an increase in activity of NR will obviously enhance the production of amino acids thereby optimizing their condensation into proteins. Furthermore, NR is known to be highly substrate inducible and hence any factor may change NR activity by influencing the nitrate availability (Khan & Srivastava, 1998).As such, the increased NRA observed after N application may well be attributed to the optimized availability of substrate(Shah, 2008). However, besides this, various other factors also regulate the activity of NR. These include the presence or absence

of irradiation (Knypl & Krystyna, 1979), and presence of hormones, such as gibberellins, cytokinins (Roth-Bejerano & Lips, 1970), auxins (Ahmad & Hayat, 1999), and monochloro-indole acetic acids (Ahmad et al., 2001).

b. Enzyme Peroxidase (POX) (EC 1.11.1.7)

Influence of GA, IAA and Kinetin on the activity of enzyme peroxidase at different leaf stages of *B. spectabilis* is depicted in table and fig. no.10.2. It is evident from the figure that the activity of enzyme peroxidase is enhanced in all the four stages of leaf development due to application of GA, IAA and kinetin growth regulators.

In all the stages of leaf development an increase in the level of enzyme peroxidase is shown by both GA and Kinetin growth hormone treatments, however IAA treatment does not enhance the peroxidase activity in all the stages of leaf development.

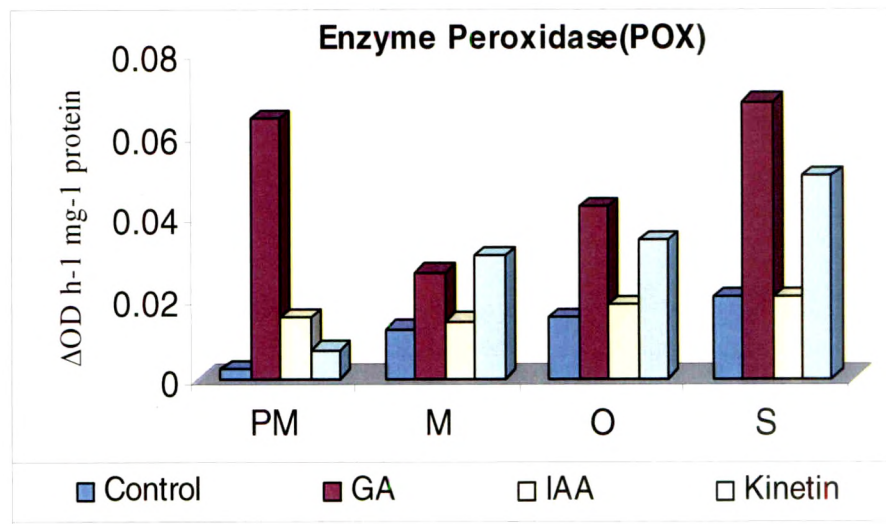
Among various plant enzymes, peroxidase is one of the most extensively studied enzymes in plants (Gasper *et al.*, 1982). Peroxidase is porphyrin enzyme catalyzing the oxidation of various substrates by hydrogen peroxidase.

Enzyme peroxidase represents antioxidative enzyme system. The enzyme found to be present in various sub-cellular components, cell membrane, nucleus etc. Nuclear peroxidase might be involved in the structural organization of chromosome using aromatic amines peroxidase catalyzes redox

Table and Fig. 10.2.

Effect of GA, IAA and Kinetin on enzyme peroxidase in *Bougainvillea spectabilis* leaves

	Control	GA	IAA	Kinetin
PM	0.002	0.064	0.015	0.007
M	0.012	0.026	0.014	0.030
O	0.015	0.042	0.018	0.034
S	0.020	0.068	0.020	0.050



PM: Premature, M: Mature, O: Onset of senescence and S: Senescence

reaction for the breakdown of H_2O_2 . Enzyme peroxidase acts on H_2O_2 and liberate nascent (O) oxygen. Peroxidase act on aromatic substances very rapidly. Peroxidase are mostly the cell wall bound enzymes. Peroxidase plays vital role in lignin synthesis and auxin metabolism (Mader and Fussl 1982; Breda *et al.*, 1993).

Vora *et al.*, (1976) observed increase in peroxidase activity due to GA treatments. The activity of peroxidase has been reported to increase with senescence advancement (Grover, and Sinha, 1985). Mukharjee and Rao (1993) showed that POD activity during maturation and in the stage of senescence of the leaves continuously increased in the *Cajanus cajan* leaves. Our results in the present investigation are quite similar to above authors. Pilet *et al.*, (1970) stated that peroxidase is composed of a number of isozymes which are capable of catalyzing several different types of oxidative reaction including oxidation of hormones. Fruits and vegetables may also contain peroxidases (EC 1.11.1.7) which can contribute to or generate browning-like reactions (Vamos-Vigyazo, 1981, Mayer, 1987; Harel, 1979). Peroxidase activity gradually decreased during the development periods, while during the periods of fruit ripening it increased (Aydin and Kodioglu, 2001).

c. Enzyme Polyphenol Oxidase (PPO) (EC 1.10.3.2)

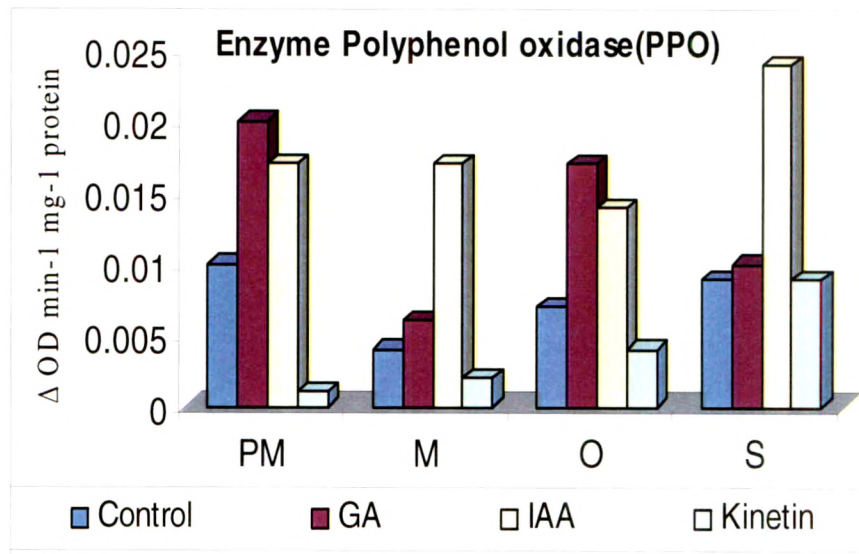
Effect of GA, IAA and Kinetin on the activity polyphenol oxidase at different leaf stages of *B. spectabilis* is shown in table and fig. no. 10.3. It is clear from the figure that the activity of enzyme polyphenol oxidase is increased in all leaf stages due to the application of GA and IAA growth regulators. However no significant change is observed in case of kinetin application in all four stages of leaf development.

There is scanty or no information is available on the polyphenol oxidase (PPO). The activity of enzyme polyphenol oxidase (PPO) is investigated by Aydin and Kadioglu (2001). During the early stages of medlar fruits development, PPO activity gradually decreased, whereas in the post-ripening stage PPO activity increased. The increase in PPO and protein contents has an important role in reducing the astringent taste of the medlar fruits. One of the major medlar fruit quality problem is flesh browning associated with the enzyme polyphenol oxidase. Polyphenol oxidase (*o*-diphenol: oxygen oxidoreductase, E.C.1.10.3.1.) has been found in higher plants, and is responsible for enzymatic browning of raw fruits and vegetables (Mathew, Parpia 1971). Polyphenol oxidase activity gradually decreased during the development of the fruits followed then by an increase during the ripening period, whereas the activity of PPO in the first months of development was higher than in the ripening months. To our knowledge this is the first report of PPO activity in the fruits of medlar. Similar results were also obtained in other

Table and Fig. 10.3.

Effect of GA, IAA and Kinetin on polyphenol oxidase in *Bougainvillea spectabilis* leaves

	Control	GA	IAA	Kinetin
PM	0.010	0.020	0.017	0.001
M	0.004	0.006	0.017	0.002
O	0.007	0.017	0.014	0.004
S	0.009	0.010	0.024	0.009



PM: Premature, **M:** Mature, **O:** Onset of senescence and **S:** Senescence

studies (Vamos-Vigyazo, 1981; Kumar, 1987; Coseteng and Lee, 1987; Park et al., 1989; Kadioglu and Yavru, 1998). Similar results are also obtained in our present investigation. 19

d. Enzyme Superoxide Dismutase (SOD) (EC 1.15.1.1)

Influence of GA, IAA and Kinetin on the activity of enzyme superoxide dismutase at four different stages of leaf development of *B. spectabilis* is depicted in table and fig. no.10.4. It is evident from the figure that the activity of enzyme superoxide dismutase is suppressed due to all the three growth hormonal treatment in all the four stages of leaf development.

Chloroplastic Fe-SOD and CuZn-SOD could be involved in protecting different parts and/or processes within this organelle. The compartmentalization of CuZn-SOD on the stroma-facing thylakoids suggests the prompt scavenging of the superoxide radical near the site of production (Ogawa *et al.*, 1995). The differential regulation of these two *sod* genes suggests a specific function for each of the enzymes in protecting chloroplasts from oxidative stress.

The mature polypeptide region of the tobacco chloroplastic CuZn-SOD is highly homologous with those from other plant species (for example, it shared 91.5% identity at the amino acid level with tomato chloroplastic CuZn-SOD). Based on the H₂O₂ and CN⁻ inhibition assays, two cathodic isoforms were identified as Mn-SOD and Fe-SOD, respectively. Both isoforms were 2ef

more abundant in senescent leaves. The two anodic SODs detected in the extracts of young leaves were CuZn- SODs (H₂O₂ and KCN sensitive). The activity of cytosolic CuZn-SOD (identified by overexpression of the *N.plumbaginifolia sodCc* in *N. tabacum*; D. Herouart unpublished data) decreased with increasing leaf age. Chloroplastic CuZn-SOD isoform was not detectable in protein extracts of senescent leaves.

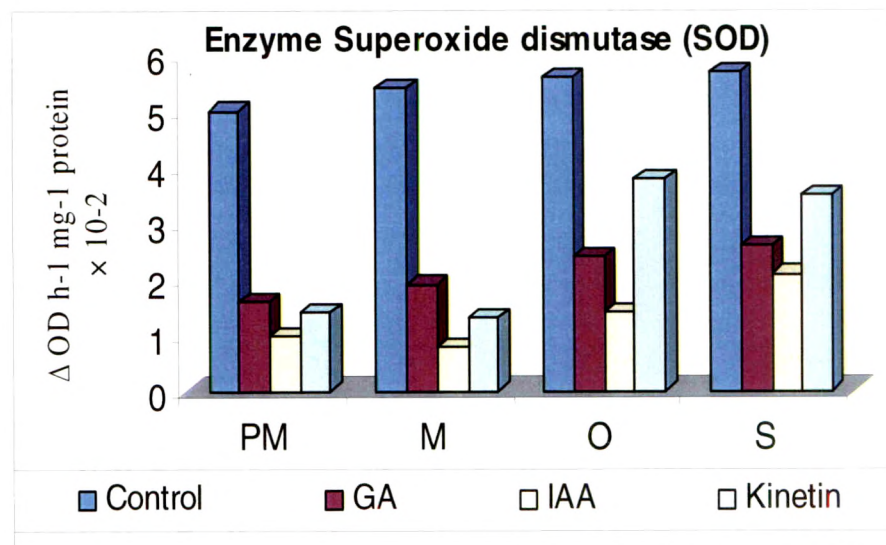
These enzymes had guaiacol peroxidase activity as was confirmed by staining the IEF gel with 4-chloro-1-naphthol. Both plastid-located SODs are developmental and regulated by most *sod* genes studied to date have been shown to be developmentally regulated. The *sodB* mRNA level was also higher in young leaves (80% higher compared to the second leaf), but the transcript remained detectable in mature and senescing leaves. The Fe- SOD activity increased with leaf age. These data suggest that Fe-SOD has a high turnover in young leaves and/or is accumulating in older leaves. Analysis of the SOD patterns of the oldest leaf and the apical part of the stem showed that in senescent leaves the major isoforms were Fe-SOD and Fe-SODS. Interestingly, the naturally occurring gradient of *sodB* mRNA, i.e. higher transcript levels in young compared to old leaves, was abolished in Norflurazon-treated plants

Previous reports have revealed some effects of hormones on *sodCp* and *sodB* expression (Casano et al. 1994, Crowell and Amasino 1991a, Perl-Treves and Galun (1991). The level of *sodB* increased during auxin and cytokinin starvation in soybean cell cultures (Crowell and Amasino 1991), whereas *sodB*

Table and Fig. 10.4.

Effect of GA, IAA and Kinetin on enzyme superoxide dismutase in *Bougainvillea spectabilis* leaves

	Control	GA	IAA	Kinetin
PM	5.00	1.60	0.97	1.40
M	5.40	1.90	0.77	1.30
O	5.60	2.40	1.40	3.80
S	5.70	2.60	2.10	3.50



PM: Premature, M: Mature, O: Onset of senescence and S: Senescence

RNA levels did not increase after dark-adapted barley leaves were treated with kinetin (Casano et al. 1994). In tomato plants treated with ethephon, *sodCp* mRNA and the chloroplastic CuZn-SOD levels increased (Perl-Treves and Galun 1991). To obtain a more general view on the effects of plant hormones on *sodCp* and *sodB* expression, we analyzed their transcriptional response to IAA, ABA, GA3, kinetin, and the ethylene precursor ACC.

In all cases, the Fe-SOD and chloroplastic CuZn-SOD activity levels were comparable to the control after 24 h of treatment. The *sodCp* mRNA levels decreased in response to all the hormone treatments (by 60% for ABA, by 50% for IAA and kinetin, and by 80% for ACC), except for the GA3 treatment that resulted in an induction of approximately 2-fold.

Both *sodCp* and *sodB* genes showed a similar response to the GA3 treatment. ABA and IAA had no effects on *sodB* expression and the *sodB* mRNA level was higher in response to kinetin (3-fold) and ACC (1.5-fold). These differences in the expression pattern again indicate different functions for *sodCp* and *sodB* in chloroplastic antioxidative system. The IAA-mediated repression of this gene might be associated with ethylene biosynthesis, because plants have been shown to have an IAA-induced ACC synthase activity (Abel et al. 1995). It is puzzling that *sodCp*, which based on its developmental expression pattern would be specific for young tissue, is expressed by the anti-senescence hormone kinetin.

In the present investigation the activity of enzyme Superoxide dismutase is increased in *B. spectabilis* during the course of senescence. Our results are similar to Hrvoje *et al.*, (2008) who stated that the increase in ROS level as well as in SOD activity during natural senescence of maple leaves.