

**RESULTS
AND
DISCUSSION**

A) PHYSICAL PROPERTIES OF LEAVES :-

Leaf area is one of the most important growth parameters which is extensively studied by several investigators. Growth and duration of green leaf area of crop determines the percentage of incident solar radiation intercepted by the crop canopy and there by influence canopy photosynthesis, evapotranspiration and final yield (Dale, 1980). Wignarajah et al. (1975) have tried to resolve this problem and their evidence indicate both the reduction in cell division and reduction in cell enlargement are responsible for reduction in leaf area of individual leaf.

Leaf area index is defined as the total leaf area over certain ground area (Sestak et al., 1971). More over it is the ability of plant stand to intercept the incident solar radiation. In agriculture field operation usually affect leaf area index other than the actual rate of photosynthesis per unit leaf area eq. sowing rate and date, rate of nitrogen fertilizer applied, irrigation treatment. Many insecticides and fumigants application and some soil cultivation treatments have their main effects on leaf area index. (Monteith and Elston, 1983). Poljakoff Mayber (1970) reported adverse effect of salinity on the expansion of leaf surface which may be associated with both a reduction in the number of leaves produced and the size of individual leaf.

In the present investigation we have observed more reduction in total leaf area in Ipomoea carnea sub sp. carnea,

than Ipomoea carnea sub sp. fistulosa.

The leaf area expansion alone does not provide a clue as to size and structure of crop canopy for evaluating leaf area index. From table No - 1 it is clear that leaf area is decreased in senescent leaves of Ipomoea carnea sub sp. carnea and Ipomoea carnea sub sp. fistulosa.

The parenchyma cells enlarge due to an increase in water content which prevent an excessive concentration of salts in cell sap (Repp, 1958). This mechanism is well developed in Atriplex species. Greenway et al (1966) and Mendora (1971) reported that Atriplex hastata exhibited increased succulence is usually taken to mean increased thickness of plant organs. Increased cellular volume/surface ratio and cellular water content are associated with increased plant succulence. Levitt (1972) reported that under natural conditions the salt content of halophytes increase gradually with increasing age and many eventually reach a toxic concentration. Many halophytes avoid this increasing concentration by increasing succulence i.e. thickness.

In the present investigation we observed the increase in thickness of senescent leaves of Ipomoea carnea sub sp. carnea and Ipomoea carnea sub sp. fistulosa. This may be due to increased moisture content (Table No - 2) to reduce the toxic effects of accumulated salts of Ca and Na (Table No - 6) in senescent leaves of both the sub species of Ipomoea carnea.

Our results depicted in Table No - 1 show that average leaf weight, leaf volume, density and moisture percent-

age increased in senescent leaves. While leaf area and dry matter decreased. The decrease in dry matter may be due to increased leaf volume, moisture percentage and mobilization of essential elements.

The average leaf weight and density increased in the senescent leaves of both the varieties of Ipomoea. This may be due to increase of sodium and calcium in the senescent leaves of Ipomoea.

TABLE - NO - 1.

Physical Properties of Green and Senescent leaves of Ipomoea carnea sub sps. carnea and fistulosa

| Plant Material | Leaf Stage | Average leaf weight in g. (A) | Average leaf area in cm ² (B) | Average leaf thickness in cm (C) | Average leaf volume cm ³ (BC) | Density A/BC ³ g/cm ³ |
|--|------------|-------------------------------|--|----------------------------------|--|---|
| <u>Ipomoea carnea</u> sub sp. <u>carnea</u> | Green | 2.450 | 86.90 | 0.018 | 1.56 | 1.57 |
| | Senescent | 2.765 | 79.92 | 0.020 | 1.59 | 1.73 |
| <u>Ipomoea carnea</u> sub sp. <u>fistulosa</u> | Green | 2.850 | 137.30 | 0.016 | 2.19 | 1.30 |
| | Senescent | 3.240 | 127.20 | 0.018 | 2.29 | 1.41 |

B) ORGANIC CONSTITUENTS.**1) MOISTURE PERCENTAGE AND RELATIVE WATER CONTENTS (R.W.C.) :-**

It is found that there is an increase of moisture percentage in the aging leaves of both the sub species of Ipomoea carnea (Table - No - 2). 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 concentration 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.

It was found that there is an increase in moisture percentage in the aging leaves of the mangroves, (Jamale, 1975). Chavan (1980) in ragi, Karadge (1981) in Portulaca oleracea, Deshpande (1981) in P. peognpea and Gujar (1983) in Tobacco have also reported an increase in moisture contents due to salination. It may be stated that salt rich environment causes increased water content in French bean which may have dilution effect on toxic as well as accumulated essential elements.

Our results (Table No - 2) also indicated the increase in moisture content and relative water content (RWC) in

Ipomoea carnea sub sp. carnea, and Ipomoea carnea sub sp fistulosa. This increase in moisture content brings about the dilution effect of an accumulated elements in the senescent leaves of Ipomoea.

2) TITRATABLE ACID NUMBER (TAN) :-

The organic acids represent an important class of metabolites in all living organisms and TAN gives general idea about their level. According to Strogonow et al (1970) organic acid like malic acid plays a protective role in plants subjected to salt stress. Different roles are attributed to organic acids in plant metabolism. Although organic acids play an important role in plant metabolism however not much attention has been paid to the fate of compounds during the plant growth and development and very few reports are available mainly about their levels in senescent tissue. Mishra and Biswal (1973) have indicated that in senescent leaves the hydrolysed products accumulate and may be partly converted into organic acids.

Organic acid level is correlated with the respiration because organic acids and keto acids are the products of TCA cycle. According to Ackerson and Younger (1975) the organic acid content is increased in Bermuda grass due to salinity which may increase salt tolerance mechanism. Nimbalkar and Joshi (1975) have reported the slight increase in TAN values in senescent leaves of sugarcane (CO - 740). This increase in content of organic acids such as malic acid is the main reason for higher value of TAN. The analysis of TAN values of first leaf tissue of groundnut, Rane (1991) showed that different developmental stages show great fluctuation in organic acid level during this course of leaf ontogeny. The TAN value

increases upto 60 to 70 days of leaf development in cultivars JL-24 and TMV - 10 respectively. TAN increases up to 90 days of leaf growth but there is again steady increase in TAN up to 120 days and maximum TAN values were recorded in 120 days old and this was due to increase in hydrolytic activity and respiratory turn over at this stage. According to Nalawade and Chavan (1991) the values of TAN during different growth stages of niger leaves are recorded in which the organic acids steadily increases as the growth proceeds.

Gokhale et al (1984) have observed a higher content of TAN in senescent leaves of Catharanthus roseus. Similarly Rangnekar (1975) and Bartakke (1977) have recorded the highest TAN values when maximum accumulation of calcium in the leaves of tomato and Aloe barbadensis under NaCl treatment. However Jamale (1975) and Karadge (1985) have observed low content of organic acids in senescent leaves of mangroves and Portulaca respectively.

In the present investigation we observed increase in TAN in senescent leaves of Ipomoea carnea sub sp. carnea and Ipomoea carnea sub sp. fistulosa.

The increased TAN can be corelated with the accumulation of calcium in the senescent leaves. These results are similar to the results found in tomato and Aloe by Rangnekar (1975) and Bartakke (1977) respectively.

TABLE NO - 2.

Moisture percentage, Dry matter production, Relative water content and TAN in Green and Senescent leaves of Ipomoea carnea :-

| Plant Material | Leaf Stage | Moisture Percentage | Dry matter Production in % | Relative Water Content | Titratable Acid Number |
|---------------------------|------------|---------------------|----------------------------|------------------------|------------------------|
| <u>Ipomoea carnea</u> sub | Green | 73.86 | 26.14 | 57.13 | 21.40 |
| | Senescent | 76.09 | 23.91 | 62.13 | 25.20 |
| <u>Ipomoea carnea</u> sub | Green | 69.77 | 30.23 | 58.20 | 19.30 |
| | Senescent | 73.86 | 26.14 | 61.09 | 23.90 |

3) CHLOROPHYLLS :-

There are several reports about the decline in chlorophylls content during senescence of detached leaf segments. Panigrahi and Biswal (1979) reported that the decrease in contents of total chlorophylls in isolated chloroplast of sunflower in continuous darkness. Fletcher and Osborne (1965) and Fletcher (1969) reported that during senescence there is decline in level of chlorophylls and degradation of proteins and nucleic acids. Dyer and Osborne (1971) reported that leaves of plant approaching senescence, the synthesis of nucleic acids and protein become progressively less and total levels fall. Quantitative change in the pigment composition are also found in senescing leaves. Sestak (1977), some species these appears to be a preferential loss of chlorophyll associated with PSE. This preferential loss of PS-I chlorophylls may be due the observed breakdown of stroma thylakoids before those of grana as reported by Ljubescic (1968) and Wolinska (1976). Endelman and Schoolar (1969) suggested that light is a major factor in chlorophyll destruction in sugarcane leaf tissue. Since the sugarcane leaf discs retained more chlorophylls in dark and lost them rapidly in the light Srichandan (1989) studied loss of total chlorophylls in isolated chloroplast incubated in light and dark. Choe and Thimann (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 the relationship between chlorophyll content and the rate of photosynthesis is equivocal. According to these workers light induced rapid degradation of chlorophylls which may be due to inability of pigments to dissipate these absorbed light energy which results in their irreversible photodestruction.

Lalitha et al (1987) studied leaf senescence in cotton leaves and reported that the decline of chlorophyll a + b was 29, 34, and 40% of control, after 24, 48 and 72 hours of senescence in dark respectively as compared to 21, 39, and 89% respectively in light. According to Begam Hasena Hena and Choudhuri (1993), the chlorophyll content in both Hydrilla and Ottelia decreased at a much faster rate in the light than in the dark, whereas Commelina Chlorophylls loss took place at a slower rate in light than in darkness during senescence. The chlorophyll a/b ratio declined more in darkness than in light.

Senescence is influenced by some growth hormones like kinetin and it is retarded by GA and IAA. Light has been reported to increase the action of cytokinin in detached leaves. Kimwag (1984) studied the hormonal changes with leaf senescence in cotton and observed that definite drop in free IAA below its initial level occurred on 24th day when most of leaf protein and chlorophylls were already broken down. GA seems to be the most effective at stages when the endogenous GA is low (Fletcher and Osborne 1965).

In the present investigation we estimated the

chlorophylls in the leaves of Ipomoea carnea Sub. sp. carnea, and Ipomoea carnea sub sp. fistulosa in green, senescent and in hormone treated leaves and the results are depicted in Table - 3 and 4.

The results clearly indicate that the chlorophylls are declined in the senescent leaves of both the sub species of Ipomoea carnea. However the treated leaves with growth harmones like GA, IAA and kinetin show retension to decrease in chlorophylls resulting in delaying of senescence.

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4) CAROTENOIDS :-

The carotenoids play a secondary role during the process of photosynthesis and general trend of content of carotenoids during leaf senescence is found to be 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. Tetley and Thimann (1974) 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. Panda and Biswal (1989) found that chlorophylls and carotenoids are degraded but carotenoids are degraded faster than chlorophylls. However in case of detached barley leaves. Biswal and Mohantly (1976) found that chlorophylls are degraded much faster than carotenoids.

Although several workers have found that continuous light causes more degradation of carotenoids than continuous darkness. Rane (1991) noticed the carotenoids loss in groundnut leaves is more pronounced under continuous darkness than under natural light. It is also observed that the carotenoids content decreased during leaf senescence is comparatively significant in groundnut leaves during induced leaf senescence.

Our results show the accumulation of carotenoids in senescent leaves of Ipomoea carnea sub sp. fistulosa, and

Ipomoea carnea sub sp. carnea.

However there are very few reports regarding the hormonal effect on carotenoid contents. In the present investigation it is observed that carotenoids are decreased in treated leaves with GA, IAA and kinetin in both the sub species.

It has been found that the treatment of GA and IAA accelerated the breakdown of carotenoids as compared to the control. However the kinetin retarding the breakdown rate of carotenoids and delaying the senescence in both the sub species of Ipomoea carnea.

5 POLYPHENOLS :-

Polyphenols are commonly known as tannins. They take part in growth metabolism and act like phytohormones.

Our results depicted in Table No - 3 indicate that there is a significant accumulation of polyphenols in the senescent leaves of both the species of Ipomoea of the present investigation. However the leaves of both the species treated with GA, IAA and kinetin show the decline of polyphenols. This decline in polyphenols is quite significant in kinetin treated leaves. Our results suggest that the polyphenols breakdown rate is faster in kinetin treated leaves than that of GA & IAA treated leaves of Ipomoea.

There are several reports regarding the accumulation of polyphenols in salt stress condition and in Impatiens balsamia under water stress condition (Todd et al, 1974). But sometimes drought may decline the polyphenol content (Magdum 1984).

Lepreton and Menerat (1964) stated that these constituents are abundant in vascular plants and they act as sexual hormones, as growth factors, as sensitizers in sythen-
sis or other phytobiological phenomenon. In case of higher plants the regulatory property of some polyphenols and their precursors is evidenced by their ability to cause leaf abscission (Kefeli 1978). Tomaszewska (1964) reported that the cinnamic acid accelerated leaf abscission and some monoxyphenols cancelled out the retarding action of IAA on leaf abscission.

According to Finkle (1967) the mode of action by which the phenolic acids effect the growth of plants is that they inhibit an enzyme that oxidatively destroys IAA and thus its hormonal effect.

Our results of hormonal treated leaves show the stimulation of polyphenol breakdown however accumulation of polyphenols is significant in untreated and senescent leaves of both the sub species of Ipomoea carnea.

Analysis of the data
data properly

TABLE NO. 3

Chlorophylls, Carotenoids and Polyphenols in Green and Senescent leaves of Ipomoea carnea.

| Plant Material | Leaf Stage | Chlorophyll - a | Chlorophyll - b | Total Chlorophyll a + b | a/b ratio | Carotenoids | Polyphenols |
|--|------------|-----------------|-----------------|-------------------------|-----------|-------------|-------------|
| <u>Ipomoea carnea</u> sub sp. <u>carnea</u> | Green | 90.16 | 59.90 | 150.00 | 1.5 | 24.0 | 0.85 |
| | Senescent | 27.71 | 22.65 | 50.35 | 1.2 | 26.0 | 1.70 |
| <u>Ipomoea carnea</u> sub sp. <u>fistulosa</u> | Green | 84.49 | 56.50 | 140.90 | 1.4 | 22.0 | 0.80 |
| | Senescent | 22.36 | 16.52 | 38.88 | 1.3 | 24.0 | 1.65 |

Values of chlorophylls, carotenoids are expressed in mg/100g fresh leaves.

Values of polyphenols are expressed in g/100g fresh leaves.

TABLE NO. 4

Effects of growth hormones on Chlorophylls, Carotenoids and Polyphenols in the leaves of Ipomoea carnea, Jacq.

| Plant Material | Treatment | Chlorophyll - a | Chlorophyll - b | Total Chlorophylls a + b | a/b ratio | Carotenoids | Polyphenols |
|--|-----------|-----------------|-----------------|--------------------------|-----------|-------------|-------------|
| <u>Ipomoea carnea</u> sub <u>sp. carnea</u> | Control | 90.16 | 59.90 | 150.0 | 1.5 | 24.0 | 0.850 |
| | G.A. | 113.50 | 67.70 | 181.2 | 1.6 | 18.0 | 0.550 |
| | I.A.A. | 102.20 | 60.98 | 163.1 | 1.6 | 14.0 | 0.350 |
| | Kinetin | 83.14 | 68.00 | 151.1 | 1.2 | 22.0 | 0.312 |
| <u>Ipomoea carnea</u> sub <u>sp. fistulosa</u> | Control | 84.49 | 56.50 | 140.9 | 1.4 | 22.0 | 0.800 |
| | G.A. | 108.60 | 58.59 | 161.1 | 1.8 | 16.0 | 0.412 |
| | I.A.A. | 90.84 | 54.16 | 144.9 | 1.6 | 12.0 | 0.250 |
| | Kinetin | 78.14 | 58.84 | 136.9 | 1.3 | 20.0 | 0.212 |

Values of chlorophylls, carotenoids are expressed in mg/100g fresh leaves.

Values of polyphenols are expressed in g/100g fresh leaves.

C) INORGANIC CONSTITUENTS

1) SODIUM (Na⁺) :

It is an important macronutrient which control plant growth and development. Joshi and Mishra (1970) observed greater content of Na in senescent leaves of Clerodendron inerme. According to them accumulation of Na⁺ and Cl⁻ in the senescent leaves might have affected the anabolic process particularly photosynthesis. Hyder (1971) noted similar results in Citrus leaves. He observed that K⁺ concentration was higher than Na⁺ and Cl⁻ contents in young leaves but when the leaves obtained adult size, there was inverse trend between Na⁺ and K⁺ contents.

The exact role of Na⁺ in plant metabolism is not well defined. But it was believed to be non-essential element for glycophytes. Epstein (1972), Brownwell and Crossland (1975) have indicated that C₄ plants need more Na⁺ than C₃. It is suggested that accumulated sodium along with chloride may be toxic to the leaf metabolism, (Joshi and Mishra, 1970).

Ambike and Karmarkar (1975) have reported a significant increase in Na⁺ and Cl⁻ in the leaves of Kalanchoe pinna-ta during senescence. They suggested that the high Na⁺ accumulation accounts for the corresponding decrease in organic acids. Hocking et al (1980), Waughman and Ballamy (1981), Pathan (1982), Gokhale et al (1984) reported similar trends. Increase in sodium content due to salinity was reported by several workers.

Bhivare (1986) studied P. vulgaris and indicated that higher content of Na in the senescent leaves of the plant are correlated with increased hydration, decreased K content and disturbed photosynthetic carbon metabolism. While Upadye (1986) reported significant accumulation of monovalent cation in the senescing and senescent leaves of T.monogyna and D. Viscosa that Na⁺ content in young developing leaves of both plants is increased dramatically during senescence. According to Naidu and Swamy (1996) the leaf sodium concentration increases slightly throughout the life span of leaves until senescence.

Our results depicted in Table No.5 show increase in sodium content in senescent leaves of both sub species. This accumulation of Na requires dilution by hydration to reduce the toxic effects on metabolism.

The results of leaf treatment with GA, IAA and kinetin show the decrease of Na content as the hormonal effect. This may be due to the stimulation of Na metabolism and acceleration of senescence in both the sub species of Ipomoea carnea.

2) POTASSIUM - (K⁺) :

According to Soni et al (1970) potassium is indispensable for plant growth and it is withdrawn from the senescent leaves. Potassium is monovalent cation required by the plant for many metabolic processes and osmotic regulation (Okenenko et al 1978). The principal role of potassium is that of an activator of numerous enzymes. Suelter (1970) has listed not less than 58 enzymes which require monovalent cations for maximal activity. Potassium is also involved in mechanism of ATP generation. It also major osmotically active component in plant cells contributing to cell turgor and enhancing the capacity of 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 old leaves to young growing organ. Dwivedi (1988) reported that potassium increases oil and protein content and energy value of ground-nut kernel. However effect of potassium deficiency on CO₂ fixation and translocation in groundnut was studied by Basha and Rao (1981) and it was noticed that K⁺ deficiency had a direct negative effect on translocation.

A decline of potassium status of senescent leaves has been noticed in several legume species like P. geonpea and horsegram (Deshpande, 1981 and Nigwekar 1988). This trend was noticeable in experiments of Waughman and Ballamy (1981) with 21 plant species. Bengtts Som et al (1983) studied uptake and distribution of potassium in Cucumber leaves of different

age and noticed that K^+ concentration decreased with increasing leaf age.

Our results also show that there is considerable decrease of K content in senescent leaves of both sub species suggesting the mobilization of K. However there are no reports regarding the effect of hormonal treatment on mineral status.

The GA and IAA treated leaves of both the sub species of Ipomoea carnea, Jacq maintain the higher level of K than the control and Kinetin treated leaves. This higher level of K in treated leaves is delaying the senescence.

POTASSIUM/SODIUM RATIO :

The potassium/sodium ratio (Table No.- 4) declined in both Ipomoea carnea sub species. Janardhan et al (1979) have demonstrated that salt tolerance of crop varieties is characterised by higher potassium/sodium ratio.

Dekock (1964) reported alternation in the ratios of different elements in the senescent leaves of Zea mays. According to him the ratio of various elements are indication of metabolic status of plant. They are metabolically interconnected and change in one of them alters other automatically. Bhivare (1984) reported that Na and K withdrawn from senescent leaves of cultivars of Phaseolus vulgare and Na/K ratio is decreased in the senescent leaves of the cultivars. It is evidenced that this changed ratio of the elements affect normal working of TCA cycle causing accumulation of organic acids. According to Bhivare (1984) such imbalance of mineral nutrients may also affect products of photosynthesis in senescent leaves of the cultivars.

From table No. - 4 it is clear that K/Na ratio is decreased in senescent leaves of both varieties of Ipomoea carnea. It is due to the withdrawal of K and accumulation of Na in the senescent leaves. However the G.A. and IAA treated leaves of both the sub species of Ipomoea carnea, maintain high levels of K resulting in higher K/Na ratio.

TABLE NO. 4

Effect of G.A., IAA and Kinetin treatment on Na, K and K/Na ratio in Ipomoea carnea, Jacq.

| Plant Material | Leaf Stage | Sodium Na | Potassium K | K/Na Ratio |
|--|------------|-----------|-------------|------------|
| <u>Ipomoea carnea</u> sub sp. <u>carnea</u> | Control | 0.140 | 1.46 | 10.4 |
| | G.A. | 0.100 | 1.66 | 16.6 |
| | I.A.A. | 0.080 | 1.52 | 19.0 |
| | Kinetin | 0.120 | 1.25 | 10.4 |
| | Senescent | 0.161 | 1.36 | 8.5 |
| <u>Ipomoea carnea</u> sub sp. <u>fistulosa</u> | Control | 0.132 | 1.44 | 10.9 |
| | G.A. | 0.100 | 1.60 | 16.0 |
| | I.A.A. | 0.090 | 1.54 | 17.1 |
| | Kinetin | 0.125 | 1.22 | 9.8 |
| | Senescent | 0.155 | 1.28 | 8.3 |

Values are expressed in g/100 g dry weight of leaves.

3) CALCIUM :

Loneragon and Snowball (1969) reported that calcium is extremely essential for growth of the young organs but it prevents its redistribution from the senescent leaves to young leaves. There are several reports of increase in calcium concentration in senescent leaves. Larkum (1968) concluded that chloroplast acts as a site of calcium accumulation. Amonkar and Karmarkar (1978) reported that calcium content is relatively more in senescent than mature or young leaves of Salvadora persica. Waughman and Bellamy (1981) analysed calcium content in leaves of 18 plant species and they reported the increase in calcium percentage in old leaves. In all 18 sps calcium content is the highest. Zajara and Kubjatko (1979) reported that when Wheat leaves aged their Ca content increased. Calcium was found in organic and inorganic compounds and was concentrated in cell walls. Hazel and Halliwell (1984) reported that Ca content of chloroplast isolated from pea (Pisum sativum) plant increases markedly with age of plants from which chloroplast are extracted. When Stocking and Ongar (1962) isolated chloroplast from calcium sufficient tobacco & bean leaves by monaqueous method to prevent loss by leaching during isolation procedure. They found that the chloroplast contained about 60% of total leaf calcium in terms of requirement in higher plants. Calcium is classified as secondary nutrients. In plants calcium exists in different forms. It is absorbed to indiffusible ions such as carboxylic, phosphorylic and phenolic hydroxyl groups. It is present in calcium ox-

alate, carbonates and phosphates.

The calcium functions as metallic enzyme activator, membrane stabilizer and in cell wall structure. An important function of calcium is to stabilize membrane and selective ion uptake in cells.

There are few attempts to study the levels of foliar calcium during different growth stages in pea nut foliage (Reid, 1976 and Hallock, 1964). Reid (1956) observed that there is no definite pattern of calcium accumulation in leaves upto 10 weeks but after that upto harvest the calcium percentage increased steadily. According to Naidu and Swamy (1996) the concentration of calcium increased throughout the life span.

The results of the present investigation show that there is considerable increase in the Ca^{++} content in the senescent leaves of both sub species of Ipomoea carnea. Increased Ca contents in the senescent leaves indicate the breakdown of chloroplast and their metabolites and less mobilization of this cation. However the G.A., IAA and Kinetin treated leaves show the decline of Ca content which suggest that Ca is either properly utilized or mobilized as result of hormonal stimulation. The treatment cause the delay of senescence.

4) MAGNESIUM (Mg⁺⁺) :

Magnesium is a constituent of chlorophyll molecule and a metallic cofactor of many enzymes. Magnesium also essentially functions as a bridging element for the aggregation of ribulose sub unit. Mg⁺⁺ is also required RNA polymerases and hence for the formation of for RNA in the nucleus. Most of the enzymes promoted by Mg⁺⁺ are categorized by general type of reaction to which they confined such as transfer of phosphate eg. Carboxylase. The synthesis of ATP (ADP + ip) has an absolute requirement for Mg⁺⁺ as bridging component between adenosine disphosphate and enzyme. Another key reaction of Mg⁺⁺ is modulation of RuBP carboxylase in 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 chloroplasts. Mg⁺⁺ is required for enzymes of ammonia assimilation like glutamine and glutamate synthetase (O Neal and Joy 1974).

A high proportion of total Mg⁺⁺ is involved in the regulation of cellular pH and carbon anion balance (Clarkson and Hanson, 1980). Chlorosis of fully expanded leaves is the most visible symptoms of Mg⁺⁺ deficiency which may be assumed to have a corresponding detrimental effect on carbohydrate supply to the root nodules and thus on N₂ fixation rate.

The change of Mg⁺⁺ during the course of leaf development and senescence has been investigated by Amonkar and Karmarkar (1978). Based on the observation with Salvadora

persica leaves and suggested that there is withdrawal of Mg^{++} ions from senescent leaves. Adamski and Bieganska (1980) analysed Mg^{++} content from Urtica dioica L. leaves at different growth stages and they reported that there was no difference in Mg^{++} content in young and old leaves.

Wuaghman and Bellamy (1981) studied Mg^{++} levels in mature and old leaves of 21 plant species and they noticed a lowering of Mg^{++} content in aged leaves. Thus decline was very much prominent in species like Hordeum vulgare, L. Where the mature leaves contained 3.5 times more Mg^{++} than old leaves.

According to Naidu and Swamy (1996) the magnesium concentration of leaves exhibited similar to calcium concentration that it increased throughout the life of the leaves.

Our results depicted in Table No. 6 show the similar trend of decline of Mg content in the senescent leaves which suggest the withdrawal of Mg from senescent leaves. The treated leaves of sub species of Ipomoea carnea, with G.A. and IAA show increased Mg content. This suggests that the GA and IAA maintain the level of Mg and delaying the senescence.

5) IRON (Fe+2) :

Iron plays an important role as a component of many enzymes which catalyse the metabolism of plant. Most of the Fe enzymes belong to the metallic enzyme group in which iron is firmly bound to protein. Frequently iron is chelated by or attached to small molecule called the prosthetic group. Peroxidase, catalase or cytochrome oxidase all contain iron bound in a heme group. This iron has affinities with these oxidase activities. According to Grog (1987) iron involved in respiration, photosynthesis, nitrate to nitrite reduction and nitrogen fixation. The role of iron in the functions of RNA or DNA must also be considered since decreases of DNA per protein in Fe deficient plant leaves have been reported by Kessler (1955).

Among various microelements the iron is paid a great attention by plant biochemists due to its key role in leaf metabolism. It is the photosynthetically available form of iron and fraction which undergoes responsible Fe-III and Fe-II oxidoreduction in chlorophylls content is the most obvious visible symptoms of iron deficiency. The common precursor of chlorophylls and hence synthesis of aminolevulinic acid and rate of ALA formation is controlled by iron or magnesium as the central atom of tetrapyrrole leads to the formation of heme co-enzyme or Mg protoporphyrin respectively. It is well established fact that iron is also required for the information of protoporphyrin in an iron protein which catalyses the oxidative decarboxylation of Mg protoporphrin (Chereksin and Cas-

telfronco, 1982). When Fe²⁺ deficiency increases, the chloroplast declines and rate of photosynthesis decreases but not per unit chlorophylls (Terry 1980).

According to Kinnan (1977) a part of Fe⁺⁺ retained in old leaves or retranslocated to new leaves at early growth stages. Chavan and Patil (1980) reported that the Fe concentration increased in aged leaves of Achras Sapota during different stages. According to them increase was depleted during fruit set. Waughman and Bellamy (1981) studied the movement of cation in some plants prior to leaf senescence and they suggested that some species may avoid accumulatory high concentration of heavy metal by selectively translocating potentially toxic cations into leaves prior to or during senescence and thus losing them during leaf abscission. Waughman and Bellamy (1981) have observed the accumulation of iron during leaf aging in 20 plant species.

The results of the present investigation show the decline in Fe content in the senescent leaves of both the sub species of Ipomoea carnea. Iron is required in many metabolic activities like chlorophylls synthesis and loss of Fe in senescent leaves suggest its withdrawal from senescent leaves. The treated leaves with G.A., IAA and Kinetin show no significant change in the Fe content and maintain its high level causing delaying of senescence of both the sub species of Ipomoea carnea.

6) MANGANESE (Mn⁺⁺) :

There are few attempts to determine the fate of Mn during leaf development and senescence. Mukharjee (1969) analysed the microelement composition of sugar cane leaves during their growth and senescence & they reported that peak concentration of Mn⁺⁺ occurred at juvenile phase of leaf growth. The analysis of (Waughman and Bellamy, 1981) the mature and senescent leaves of 21 plants species revealed that there was decline in Mn level during leaf aging, while Gokhale et al (1984) reported that in Catharanthus roseus Mn content increased considerably with expansion and maturity of leaves upto presenescent state but later on slight decline in Mn content at senescent stage. Bhivare and Nimbalkar (1984) have noticed no differences in Mn⁺⁺ content in mature and senescent leaves of beans. Mishra et al (1976) have observed paragenetic changes in Mn⁺⁺ during senescence in betal leaves.

Manganese constitutes an important micronutrients which has a role in phosphate transfer reactions and in the Krebs cycle, a number oxidases and in photosynthesis (Burke et al, 1990). It is also involved in metabolism of nitrogen, auxin and nucleic acids. Bruke et al (1990) determined plant Mn⁺⁺ and organic acid concentration in tolerant and sensitive cultivars of wheat and found that without Mn⁺⁺ the malic acid to pyruvic acid does not function. The most well known and extensively studied function of Mn⁺⁺ in green plant is its involvement in photosynthetic O₂ evolution.

If the crops are subjected to both manganese

deficiency as well as Mn toxicity depending upon soil condition then an increase in peroxidase activity is a typical feature of Mn deficient tissue (Barakiva and Lavon 1967). Mn deficient leaves exhibit exceptionally high IAA oxidase activity (Morgan et al 1976) which might lead to enhanced auxin (IAA) degradation in the tissue. In Mn deficient cells, not only the chlorophyll content but the content of typical chloroplast membrane constituents such as glycolipids and polysaturated fatty acids are reduced (Constantopoulos, 1970).

The analysis of Mn⁺⁺ status in both the Ipomoea carnea species show that there is decrease in Mn content during the senescence. Our results shows similar trends as observed by other researchers. GA and IAA treated leaves show the stimulation for Mn absorption and its accumulation, however the Kinetin treated leaves show decrease of Mn content in both the sub species of Ipomoea carnea.

7) COPPER (Cu) :

Copper is a component of several metalloenzymes and it appears to act as intermediate electron acceptor in the direct oxidation of substrate by molecular oxygen. It is not readily mobile in the plant, although it can be translocated from older to younger leaves. It is a constituent of the chloroplast protein plastocyanin which forms part of electron transport chain linking the two photochemical systems of photosynthesis (Bishop 1966). While observation of Hallworth et al (1966) suggest that there is a specific requirement for copper in symbiotic nitrogen fixation. It is supposed that copper may be involved in leghaemoglobin synthesis. Copper is playing a role in nodulation of legumes. It plays an important role for enzyme cytochrome oxidase which is the terminal oxidase of respiratory enzyme system.

Mishra et al (1976) recorded a paragenetic changes in copper during senescence in betal leaves. According to them a fast induction of senescence by copper could be due to degrading effect on chlorophylls and interference in its synthesis. It is evident from findings that retraslocation of copper characteristics of senescing leaf tissue. According to Rane (1991) in the first leaf of both cultivars of groundnut only upto 100 days the copper status is maintained to an optimum level. This may be possibly one of the metabolic factor for apparent absence of leaf senescence in groundnut. There is decline in copper level in the old leaves (110 and 120 days).

In the present investigation it is found that there is more considerable decline in Cu content in senescent leaves of Ipomoea carnea sub sp. fistulosa variety than Ipomoea carnea sub sp. carnea. The treated leaves of both the sub species of Ipomoea carnea with IAA and GA show slight stimulation for absorption and accumulation of Cu however the Kinetin treated leaves of both the species show slight decline of Cu.

8) ZINC (Zn⁺⁺) :

In plants zinc is neither oxidised nor reduced and it functions as a mineral nutrients which are based primarily on its properties as divalent cation with a strong tendency to form tetrahydral complex (Clarkson & Hanson 1980). Zinc acts as a metal component of enzyme or as a functional, structural and regulatory cofactor of large number of enzymes. High rate of RNA activity is a typical character of zinc deficiency. A clear inverse correlation exists between zinc supply and RNA activity and also between RNA activity and protein content (Sharma et al 1982).

Ghildiyal et al (1986) have observed a decrease in protein nitrogen content and increase in free amino acids content of linseed varieties under Zn⁺⁺ deficiency which indicates that Zn⁺⁺ is playing a prominent role in nitrogen metabolism. Vesik et al (1966) showed that the possible involvement of Zn in process of photosynthesis is indicated by demonstration of prominence in chloroplast structure. The role of Zn⁺⁺ in carbohydrate metabolism is related to the photosynthesis and process of phosphorylation. Zn⁺⁺ is a constituent of many glycohytic, respiratory and several FAD and NAD dependent enzymes. So its deficiency can lead to several problems pertaining to growth and development.

Chavan and Patil (1980) studied the chemical composition of Acharas sapota L leaves and they observed that Zn⁺⁺ content was the highest in the medium aged leaves. While Menzel et al (1987) investigated the effect of leaf age on

nutrient composition of litchi and they reported that levels of Zn^{++} decreased markedly on leaf aging. Old leaves had 2-3 times less of these nutrients as compared to young leaves and there was a great decline occurred in Zn^{++} content in the mature leaves.

Our results depicted in Table No. 6 clearly show the decline in Zn content in senescent leaves of both species of Ipomoea carnea. IAA and GA treated leaves of both the species show slight increase of Zn content which may be due to stimulation caused to increase and maintain the Zn level. However the Kinetin treated leaves show decrease of Zn content.

TABLE - NO. 5

Inorganic constituents in Green and senescent leaves of Ipomoea carnea, Jacq.

| Plant Material | Leaf Stage | Sodium `Na' | Potassium `K' | Calcium `Ca' | Magnesium `Mg' | Iron `Fe' | Manganese `Mn' | Copper `Cu' | Zinc `Zn' |
|---|------------|----------------|------------------|-----------------|-------------------|--------------|-------------------|----------------|--------------|
| <u>Ipomoea</u> <u>carnea</u> sub <u>sp. carnea</u> | Green | 0.140 | 1.46 | 1.75 | 1.58 | 0.084 | 0.007 | 0.0040 | 0.0030 |
| | Senescent | 0.161 | 1.36 | 3.52 | 1.15 | 0.069 | 0.005 | 0.0036 | 0.0026 |
| <u>Ipomoea</u> <u>carnea</u> sub <u>sp. fistulosa</u> | Green | 0.132 | 1.44 | 1.86 | 1.42 | 0.077 | 0.007 | 0.0042 | 0.0032 |
| | Senescent | 0.155 | 1.28 | 3.35 | 1.12 | 0.058 | 0.006 | 0.0032 | 0.0024 |

Values expressed in g/100 g of dry material.

TABLE NO. 6

Effects of growth hormones on Inorganic Constituents in the leaves of Ipomoea carnea, Jacq.

| Plant Material | Treatment | Sodium `Na' | Potassium `K' | Calcium `Ca' | Magnesium `Mg' | Iron `Fe' | Manganese `Mn' | Copper `Cu' | Zinc `Zn' |
|---|-----------|----------------|------------------|-----------------|-------------------|--------------|-------------------|----------------|--------------|
| <u>Ipomoea carnea</u> sub sp. <u>carnea</u> | Control | 0.140 | 1.46 | 1.75 | 1.58 | 0.084 | 0.007 | 0.0040 | 0.0030 |
| | G.A. | 0.100 | 1.66 | 1.60 | 1.76 | 0.094 | 0.008 | 0.0046 | 0.0034 |
| | I.A.A. | 0.080 | 1.52 | 1.50 | 1.60 | 0.082 | 0.008 | 0.0046 | 0.0032 |
| | Kinetin | 0.120 | 1.25 | 1.94 | 1.02 | 0.073 | 0.006 | 0.0034 | 0.0024 |
| <u>Ipomoea carnea</u> sub sp. <u>fistulosa</u> | Control | 0.132 | 1.44 | 1.86 | 1.42 | 0.077 | 0.007 | 0.0042 | 0.0032 |
| | G.A. | 0.100 | 1.60 | 1.45 | 1.63 | 0.087 | 0.008 | 0.0049 | 0.0036 |
| | I.A.A. | 0.090 | 1.54 | 1.42 | 1.51 | 0.066 | 0.008 | 0.0047 | 0.0033 |
| | Kinetin | 0.125 | 1.22 | 1.82 | 1.09 | 0.053 | 0.006 | 0.0030 | 0.0020 |

Values expressed in g/100 g of dry material.

D] ACTIVITIES OF ENZYMES DURING SENESCENCE :1) PEROXIDASE : (EC 1:11:1:7.) :

The enzyme peroxidase is an indicator of respiration rate (Horovitz et al, 1968). There are several reports regarding the peroxidase activity during senescence. In case of detached tobacco leaf segments, Parish (1968) noticed increase in peroxidase activity. The activities of several enzymes either generating or decomposing O_2 or H_2O_2 were investigated during the course of senescence in detached wheat and rice leaves in light and darkness by Kar and Feierabend (1984). The increase was higher in the dark than in light. According to those workers the increased peroxidase activity accompanies the senescence of detached leaves.

Pillet et al, (1970) have reported that peroxidase can bring about oxidation of auxin and hence its increase in senescent leaves may induce hormonal imbalance. However Parish (1968) suggested that increase in peroxidase activity is one of the most reliable indicators of maturity and senescence. According to Mukherjee and Rao (1993) Peroxidase activity increased constantly during leaf maturation and much higher level was during senescence.

According to Rane and Chavan (1993) changes in peroxidase activity during senescence of detached leaf segment in darkness of groundnut cultivars TMV-10 and JL-24 showed that in both the cultivars there was continuous increase in peroxidase activity as senescence progressed.

Our results of the present investigation (Table No.7) clearly indicate that the peroxidase activity is increased during the senescence of both the sps. of Ipomoea carnea.

2) ACID PHOSPHATASE (EC 3:1:3:2) :

Acid phosphatase is one of the important enzymes of phosphorus metabolism which is involved in breakdown of several phosphates including sugar phosphates and even ATP (De Lev and Sacher 1970). Acid phosphatase has multiple molecular forms particularly with respect to possible changes during maturation and senescence of plant tissues (Baker and Takeo 1973). In certain plant tissues like leaf discs of Rhoeo discolor, acid phosphatase increased considerably (De Leo and Sacher 1970). According to Baker and Takeo (1973) the significant rise in acid phosphatase prior or during senescence of certain plant tissue is not clear and they reported that it is not general phenomenon of senescence of plant tissues. This enzyme is not involved in autolytic process during senescence. An increase in acid phosphatase involved in catabolic process during rice leaf senescence was found by Kar and Mishra (1976). Besford (1979) reported that the acid phosphatase activity in the expanding leaves was greater on a fresh weight basis than in the young fully expanded or mature leaves.

Recently Rane (1991) has observed the activity of acid phosphatase on groundnut and found that acid phosphatase activity is elevated during course of induced senescence in the two groundnut cultivars. Rane also found that the increase in acid phosphates in senescence of groundnut leaf takes place depending upon decrease in protein level during senescence. Thus during leaf senescence process acid phosphatase may play a critical role particularly with respect to retranslocation

of phosphorus.

In the present investigation we found increased acid phosphatase activity in the senescent leaves of both the species of Ipomoea carnea.

Table No- 7.

Activities of enzymes Peroxidase and Acid phosphatase in Green and Senescent leaves of Ipomoea carnea Jacq :-

| Plant Material | Leaf Stage | Peroxidase Activity | Acid Phosphatase Activity |
|--|------------|---------------------|---------------------------|
| <u>Ipomoea carnea</u> sub sp. <u>carnea</u> | Green | 1.27 | 2.43 |
| | Senescent | 2.13 | 3.75 |
| <u>Ipomoea carnea</u> sub sp. <u>fistulosa</u> | Green | 1.18 | 2.28 |
| | Senescent | 2.10 | 3.62 |

Values are expressed in $\Delta OD \text{ h}^{-1} \text{ g}^{-1}$ fresh tissue.