RESULT AND DISCUSSION

CHAPTER - III

1. GROWTH STUDIES

The effect of foliar application of salicylic acid on the various growth parameters recorded in Table No. 4 (a) (Photoplate No. 3 to 8) which included shootroot length, number of branches, number of leaves per plant, leaf area, no. of root nodules, height of plants and pod yield per plant. The fresh and dry weights of shoot and roots in both cultivars of groundnut are recorded in Table No. 6 (b).

Incase of Cv. W-44 shoot length increased successively with increase in SA concentration. In particular 50 ppm SA treatment has markedly promoted shoot growth. The root length is increased only by 50 ppm SA treatment.

Whereas in Cv. SB-11, shoots length increased by all concentrations of SA except 200 ppm SA while root length promoted by all SA concentrations except 5 ppm SA. The number of branches per plant also increased by all treatments in both studied groundnut cultivars. Similarly SA treatment increased number of leaves per plant, more significant results are obtained with 5 ppm SA in both cultivars. The number of root nodules increased with all SA doses particularly by 100 and 200 ppm SA concentrations in Cv. W-44 and SB-11. The treatments of higher concentrations of SA increased the number of pods in both the studied cultivars.

The fresh and dry weight of both cultivars shows positive response to SA. The maximum increase in fresh weight is noticed by lower concentrations of SA i.e. 50 ppm in Cv. W-44 and 5 ppm in SB-11 while reduction in fresh weight is noticed with 100 ppm SA in Cv. SB-11. The biomass is also increased due to all SA concentrations applied in the same manner of fresh weight.

Several reports have been achieved on the effect of exogenous application of SA on the growth parameters in plants. Singh and Kaur (1980) found increased yield and number of pods in mung bean treated with SA. Similar results have been recorded by Rendon (1983) in *Phaseolus Vulgaris*. The phenolic compounds such as monophenols (8 amino., 3, 6,- disulphate and naphthol) or diphenols (1, 3 dihydroxy benzene) increased seed oil content, seed yield and shelling percentage in groundnut Cv. M-37 and C-501 (Singh *et al.* 1991). Patil (1993) noticed that a single spray of 400 ppm of aspirin recorded highest dry pod yield in groundnut Cv. SB XI. Rathore (1995) studied the effects of salicylic acid (monophenol), caffeic acid (diphenol) and tannic acid (polyphenol) on groundnut Cv. Chandra, from these studies they concluded that 100 ppm tannic acid caused higher pod yield (47 g/ha) and 40 ppm SA resulting maximum linoleic acid content in seeds (23.4%). SA induces growth of shoot and roots in

soyabean plants (Guitierrez, et al. 1998). Similarly SA also increased the leaf area in sugrance plants by Dhaliwal et al. (1997) and Zhou et al. (1999) and in maize plants by Khodary (2004).

SA hastened the floral bud formation as well as pod formation. The treatment of 50 ppm SA at 24 days increased number of flowers and pods per plant, grain yield and harvest index (Kumar et al., 1999). Foliar application of Brassinosteroids, salicylic acid, naphthalene acetic acid, mepiquat chloride improved maximum grain yield and grain protein. Similarly 0.1 ppm brassinosteroid and 10 ppm triacontanol increased maximum grain yield in pearl millet (Sivakumar et al., 2002). Ghai et al. (2002) recorded that foliar application of paclobutrazol and salicylic acid at 10 and 20 mug ml-1 GLS-1 improved seed yield of Brassica napus. Khan et al. (2003) found that foliar application of salicylic acid, acetyl salicylic acid and gentisic acid increased leaf area, plant dry mass of corn and soyabean plant whereas plant height and root length remained unaffected with application of salicylates. Fariduddin et al. (2003) reported that 10⁻⁵ M SA increased pod number, 10⁻³ M and 10⁻⁵ M SA increased seed numbers per plant and total seed yield increased with 10⁻⁵ M SA in Brassica juncea. Singh and Usha (2003) noticed that dry mass of wheat under water deficit conditions was higher with SA application. Khodary (2004) studied the effect of 10⁻² M SA on counter acting the NaCl deterious effects on maize cultivars. They found that SA treatment increased the growth parameters such as shoot and root lengths, fresh and dry weights and leaf area.

Recently, Ghulam *et al.* (2007) investigated the effect of SA in 3 modes such as, seed treatment, seed treatment + foliar application and foliar spray in *Pisum Sativum* on growth parameters. They observed increased yield components in pea plants which was treated with seed treatment + foliar application of SA 10 (-4) M. Larque- Saavedra and Martin Mex (2007) reported that lower concentration of SA caused the increased plant size, number of flowers, leaf area, yield, root density and root length in horticultural plants.

It is revealed from the foregoing accounts that the SA influences the growth components such as shoot-root length in higher proportion; it can confirm its role as a phytoharmone. Similarly increase in number of branches and leaves with SA application is in agreement with earlier reports such a positive correlation increases photosynthetic capacity of crop ultimately helpful for increasing crop productivity. The nitrogen requirement of groundnut is much higher than cereals. The role of root nodules in nitrogen fixation is well known. In the present study increase in root nodules per plant with application of SA in both groundnut cultivars also correlated with our findings of nitrogen content. In addition to it the accumulation of Zn also enhanced the nodulation process in groundnut is also evident from our findings recorded in fig. 30-31.

The role of SA as an antitranspirant compound has been explained by various workers such as Aktas (2001), Agarwal *et al.* (2005) and Tuna *et al.* (2007). The induction of relative water content in leaf tissue due to SA application increases fresh weight. Similar reports also noticed in the present investigation in shoot and roots of both groundnut cultivars.

The accumulation of dry matter in plants is contributed mainly by photosynthetic activity. The photosynthates produced by these activities either utilized for various catabolic processes or accumulated as dry matter. It is evident from present investigation that both the groundnut cultivars increased dry weight with SA application.



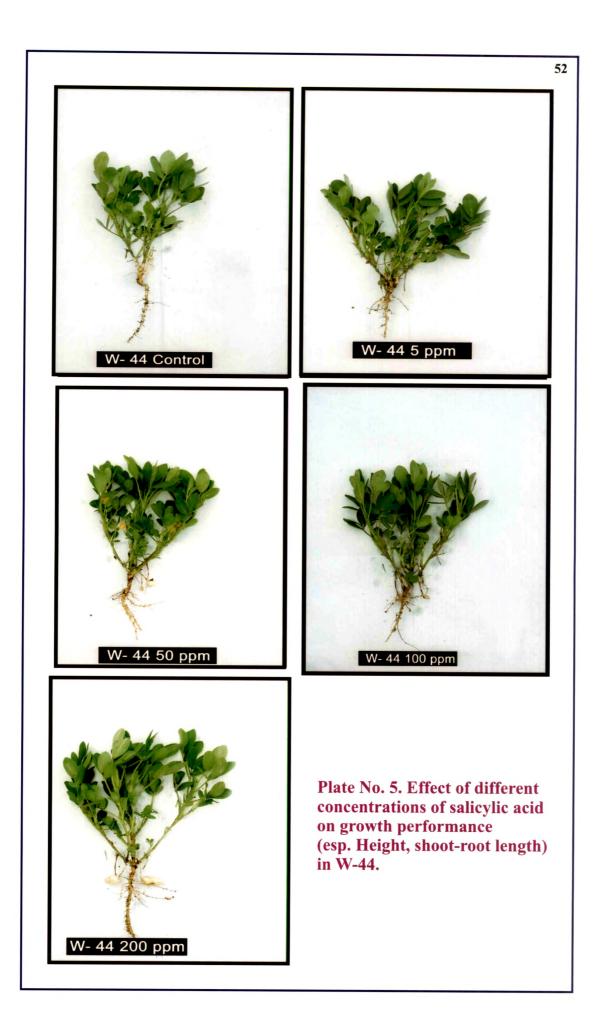


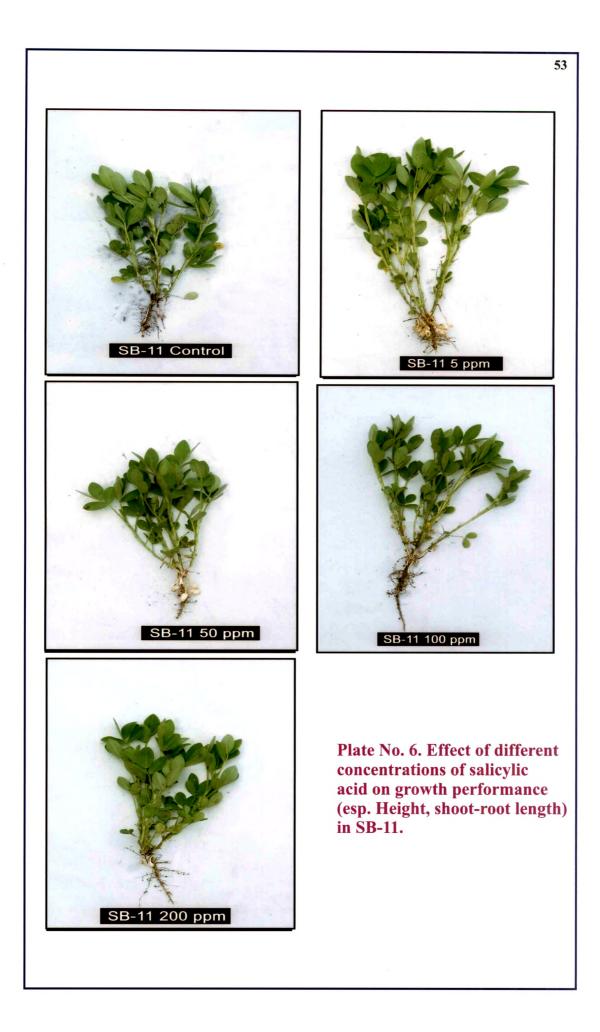
Cultivar	Salicylic Acid (ppm)	Lengths (cm)		Number of	Number of	Leaves	Number of Root	Number
		Shoot	Root	branches plant ⁻¹	Leaves plant ⁻¹	area (cm ²)	Nodules plant ⁻¹	of Pods Plant ⁻¹
W-44	Control	20.2	13.6	3	28	7.89	26	34
	5	23.2	10.5	6	55	8.38	54	23
	50	34.4	14.0	5	54	10.11	59	41
	100	33.1	12.2	5	43	10.85	65	35
	200	24.7	13.6	5	46	7.21	71	39
SB-11	Control	26.4	8.6	4	27	7.96	31	22
	5	38.6	8.4	6	48	11.70	83	17
	50	28.1	10.6	4	29	12.77	83	33
	100	33.7	16.4	6	45	12.19	86	39
	200	25.9	11.9	5	33	10.24	87	40

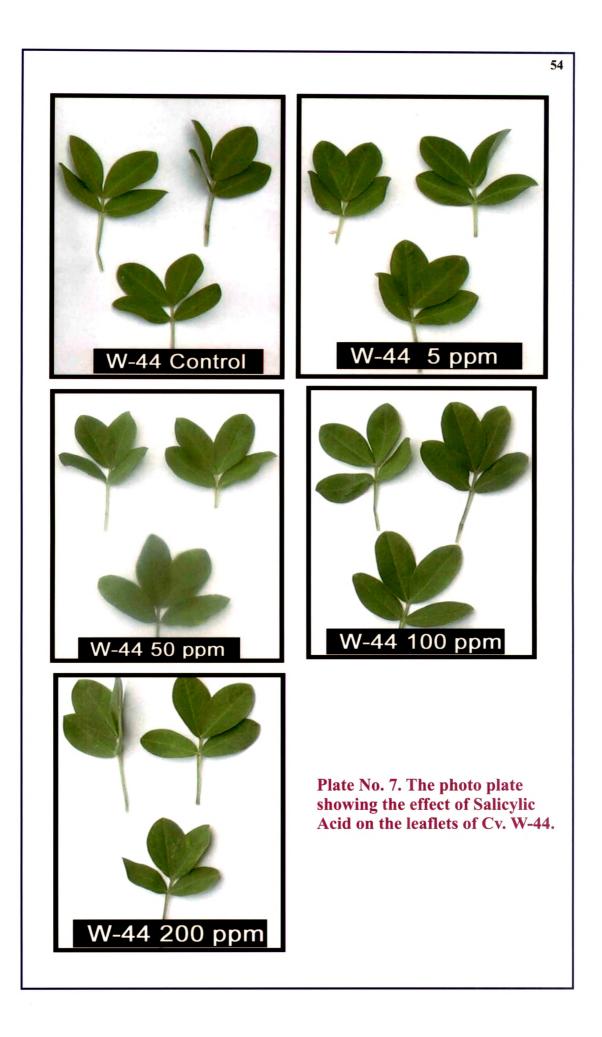
Table 4 (a): Growth characteristics of Groundnut cultivars in response to foliar application of Salicylic Acid

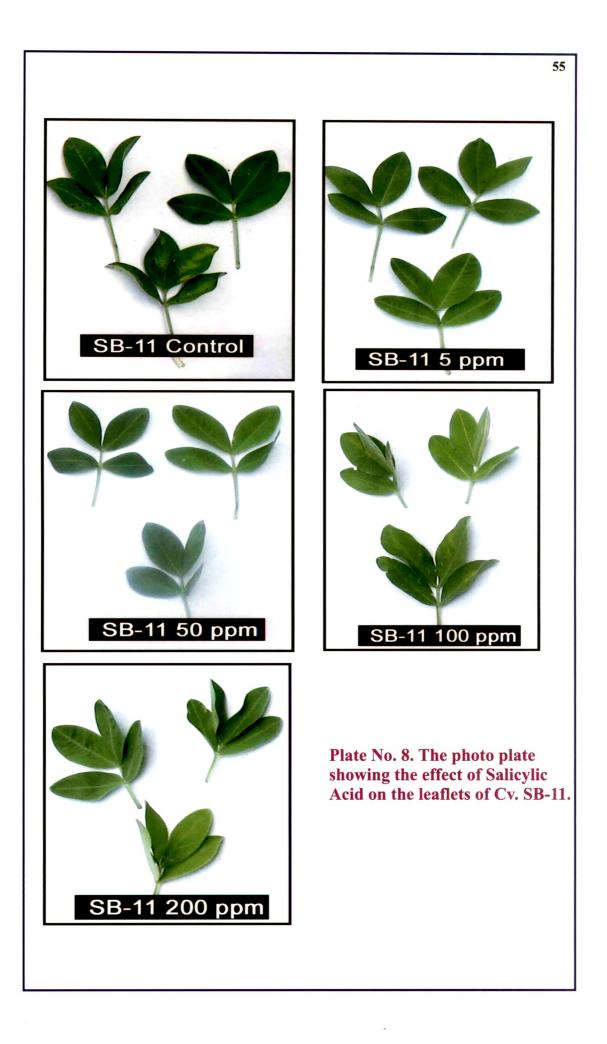
Caltinaa	Salicylic	Fresh Wei	ght g/plant	Dry Weight g/plant		
Cultivar	Acid (ppm)	Shoot	Root	Shoot	Root	
	Control	9.00	1.06	2.93	0.39	
	5	20.51	1.16	6.16	0.51	
W-44	50	22.60	1.26	7.01	0.49	
	100	15.52	0.88	4.85	0.35	
	200	19.02	1.31	5.91	0.51	
	Control	9.23	1.23	2.56	0.47	
	5	46.23	1.84	11.30	0.61	
SB-11	50	18.85	1.08	4.83	0.36	
	100	30.24	1.75	8.57	0.82	
	200	19.71	1.44	5.20	0.52	

Table 4 (b) : Growth characteristics of Groundnut cultivars in response to foliar application of Salicylic Acid









2. PHOTOSYNTHETIC PIGMENTS

a) Chlorophylls

The effect of various concentrations of salicylic acid on different photosynthetic pigments in leaves of groundnut cultivars W-44 and SB-11 is depicted in Figure 7-12. Incase of Cv. W- 44, the chlorophyll a and b content is significantly increased by 50, 100 and 200 ppm SA treatments while incase of Cv. SB-11, all the SA treatments increased the chl a and chl b content and it is noticeable with 5 and 100 ppm salicylic acid. The total chlorophylls also show responce in similar manner in both the cultivars due to SA treatments.

Chlorophylls are the most important pigments performing the process of photochemical reactions during photosynthesis. Chlorophylls occupy the most important place in the plant cells because of their involvement in harvesting of solar energy and converting it to chemical energy. Chlorophylls are basically the magnesium chelates of closed tetrapyrrole rings derived from protoporphyrin through a number of steps which together with iron porphyrin represents the end products of porphyrin metabolism in plants.

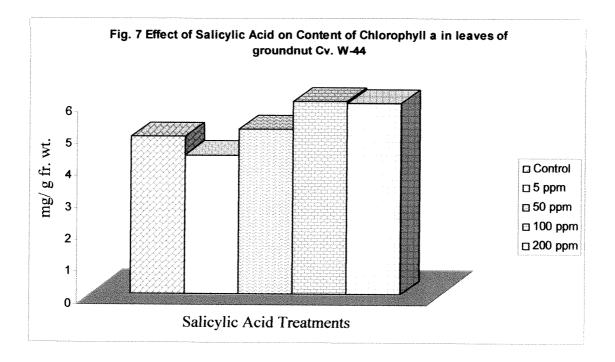
Chlorphylls are belongs to a class of lipids. Higher plants are characterised by the presence of chl. a and chl. b which are part and parcel of the photosynthetic apparatus. Among these two pigments chl a plays a key role in light reactions while chl. b plays a accessory role. Chlorophylls act as an electron gun and participated in the conversion of solar energy into chemical energy. As the chlorophylls plays a key role in light reactions, its content as well as the state of these pigments have direct influence on the photosynthetic efficiency of the plant. Induced chlorophyll a fluorescence *in vivo* reflects underlying changes in pigment composition and the electron transport through PS II chlorophyll fluorescence yield is used as a measure of photosynthetic efficiency.

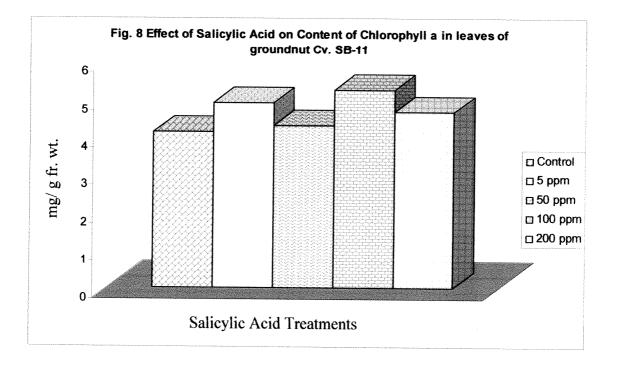
Rane (1987) observed the changes in photosynthetic pigments in leaves of two groundnut cultivars. She noticed that all franctions of chlorophyll declined with age. There are several reports are available on the involvement of salicylic acid in the photosynthesis (Rajasekaran and Balke, 1999, Janda *et al.*, 2000; Smith *et al.*, 2001., El- Tayeb *et al.*, 2005). According to Moharikar (2001) salicylic acid pretreatment reduced the chl a, and chl a/b ratio in wheat where as all treatments except 200 ppm SA increased chl a, chl. b and chl a/b ratio in moong. Ghai *et al.* (2002) showed that foliar application of 10 and 20 mug ml⁻¹ GLS⁻¹ paclobutrazol and salicylic acid enhanced chlorophyll content in *Brassica napus*.

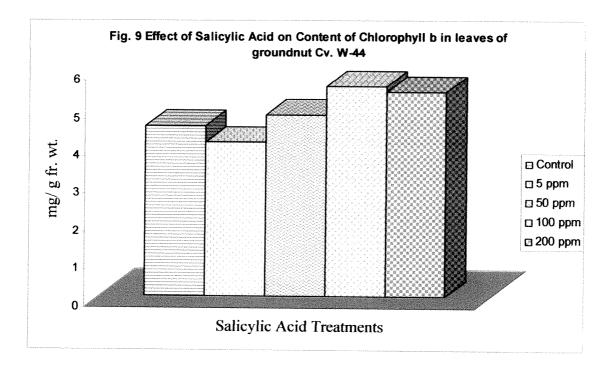
Sivakumar et al. (2002) reported that foliar application of brassinosteroid (BR), Triacontanol, Salicylic acid (SA), naphthalene acetic acid (NAA) and mepiquat chloride increased the chlorophyll content in pearl millet. Czerpak et al., 2002 found that 3- indolacetic acid (IAA) and salicylic acid increased the chl a and chl. b levels in Wolffia arrhiza. Foliar sprays of salicylic acid (SA), acetyl salicylic acid (ASA), gentisic acid (GTA) increased the photosynthetic rates of soyabean and corn. They also recorded the increased photosynthetic rates of corn in response with chronic injection of salicylic acid (Khan et al., 2003). Singh and Usha (2003) reported increase in total chlorophyll content in wheat seedlings in response to salicylic acid treatment. El-Tayeb (2005) has been found that barley grains soaking presowing in different concentrations of NaCl decreased the chl a and chl b contents in contrast to it barley grains presowing soaking in 1mM Salicylic acid caused the amelioration in photosynthetic pigments (chl a and chl b). Shi et al. (2006) studied the influence of SA on chlorophyll fluorescence (as a non-invasive method to determine the functional state of the photosynthetic machinery) before heat stress treatments, 36 h after heat stress and 24 h after recovery. They observed that foliar spray of salicylic acid increased Fv/Fm and Φ PS II after as well as recovery of heat stress. Arfan et al. (2007) indicated that 0.25 and 1 mM salicylic acid treatments increased the chl a content of wheat leaves under salt stress. Tuna et al. (2007) has been studied the effects of salicylic acid, 5 - sulfosalicylic acid (SSA) and acetyl salicylic acid (ASA) in maize plants under salinity stress. They further stated that among the studied derivatives only 1 and 2 mM sulfosalicylic acid caused induction in contents of total chlorophylls.

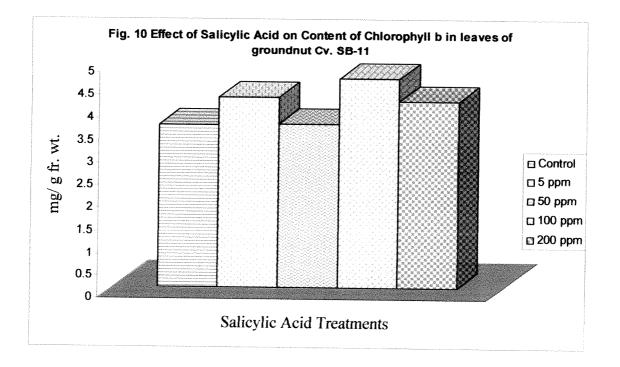
Salicylic acid also caused inhibitory action on chlorophyll contents. Anandhi and Ramanujan (1997) observed the reduction in chl. a and chl b content in blackgram Cv. CO-5 and T-9, incase of Cv T-9 chl b level was elevetated at 10 mM SA treatment, similarly total chlorophyll contents also showed to be reduced in both the cultivars. Salicylic acid caused the reduction in chlorophyll content in barley (Pancheva *et al.*, 1998).

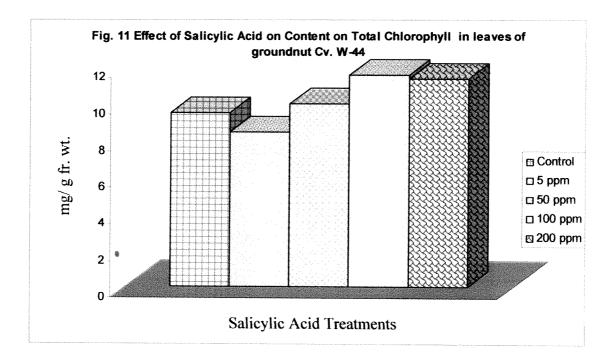
The decrease in chlorophyll content with maturation period of groundnut related with decreasing photosynthetic efficiency. However it is evident from our results that all components of chlorophylls increased by application of higher concentrations of SA in both cultivars of groundnut. These changes indicate that SA application stimulates photosynthetic activities help both the groundnut cultivars for better yield production.

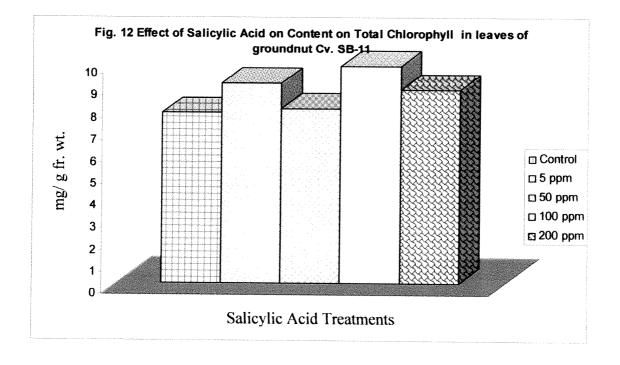












b) Carotenoids

The effect of foliar application of salicylic acid on carotenoid contents is recorded in fig. 13 and 14. It is clear from the figures that incase of Cv. W-44, among the applied SA treatments only 100 ppm SA treatment increases carotenoid content while incase of Cv. SB-11 100 as well as 200 ppm SA increased the carotenoid contents. The reduction in carotenoid content by 50 ppm SA in both groundnut cultivars is observed.

Carotenoids are accessory pigments. Carotenoids are synthesized and accumulated along with the chlorophylls in functional protein complexes (ppc) in thylakoid membrances (Siefermann-Harms, 1985). Carotenoids constitute an integral part of photosynthetic apparatus energy transfer and phototropism carotenoids are photoprotective in nature. The core complexes of PS I and PS II contains β - carotene while light harvesting chlorophyll proteins contains the xanthophylls, lutein, violoxanthin and neoxanthin (Britton, 1988). Carotenoids protect the chlorophylls against bleaching.

In higher plants, carotenoids function as antenna pigments, involved in harvesting and transmitting radiant energy with some losses to chlorophyll a molecules. Carolenoids acts as carriers of oxygen through epoxidation. It protects the chlorophylls against lethal photosynthetic oxidation by scavenging unwanted oxygen and channeling it into epoxide formation.

It is evident from earlier report by Rane (1987) that carotenoid content increases with progressive of age after 30 days of groundnut growth. SA treatments increase the contents of carotenoids and chlorophylls in maize leaves (Sinha *et al.* 1993). According to Moharikar (2001) 50, 100 and 200 ppm salicylic acid induced the violoxanthin, Anthraxanthin, lutein and Zeaxanthin levels in Wheat seedlings. Czerpak *et al.* (2002) found that salicylic acid and 3- indole actic acid (IAA) significantly increased the content of carotenoids esp. beta-carotene and lutein + Zeaxanthin in *Wolffia arrtiza*, growing on municipal tap water (poor in organic compounds). Khodari (2004) noticed the higher contents of carotenoids in SA treated maize plants than control and salt-stressed maize plants. They noticed the stimulatory effects of salicylic acid on photosynthesis and levels of pigments.

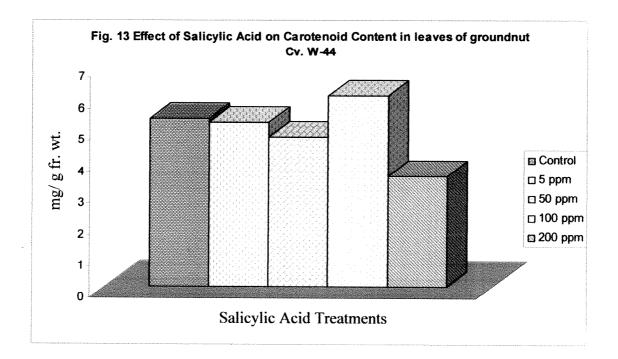
Recently Tuna *et al.* (2007) investigated the influence of salicylic acid, sulfosalicylic acid and acetyl-salicylic acid on the carotenoid contents. They recorded that among the studied derivatives of salicylic acid, 1 mm sulfosalicylic acid increased the carotenoid content in saline stressed maize plants. Similar results also recorded by Agarwal et al. (2005) in Wheat genotypes.

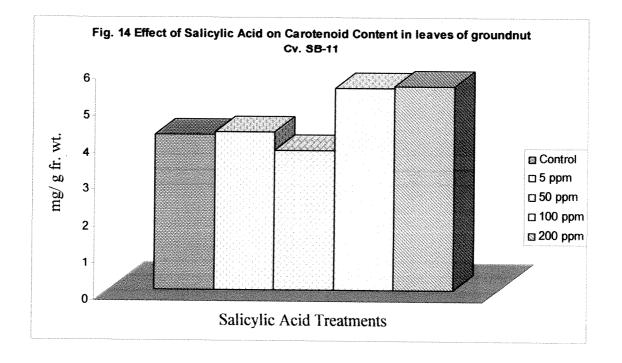
Anandhi and Ramanujam (1997) found reduction in carotenoid content with all applied salicylic acid doses in *Vigna mungo* cultivar, Co-5 and T-9.

It is evident from our observations that the response of various concentrations of SA in carotenoid content is not uniform. The lower concentrations of SA reduced cartenoid content in both cultivars but promotion in carotenoid recorded by 100 ppm SA treatment in both cultivars. Thus this dose of SA might be protected light harvesting assembalies.

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3. ENZYME STUDIES

a) Enzyme Nitrate Reductase (E.C. 1. 6. 6. 1)

The influence of exogenous application of SA on enzyme nitrate reductase (NR) in groundnut Cv. W-44 and SB-11 is recorded in fig. 16-17. It is clear from the figures that the activity of NR is increased significantly than the control except 5 ppm SA by higher doses (100 and 200 ppm) of SA in Cv. SB-11. The overall decline in activity with increase in SA treatment is observed in Cv. W-44. The effects are more distinct with higher SA treatment i.e. from 50 to 200 ppm of SA in Cv. SB-11.

The first enzyme involved in the assimilation of nitrate by higher plants is nitrate reductase (NR.) About 99 % of the organic nitrogen in biosphere is derived from the assimilation of nitrate. Nitrate is the principal source of nitrogen for most plants growing under various field conditions. Its accumulation is not injurious or potentially toxic the large variations of nitrate in cellular concentrations controlled by enzyme nitrate reductase. The NR enzyme is found to be located in the cytosol of root epidermal and cortical cells as well as in shoot mesophyll cells. NR is a metalloflavo protein catalyzes the reduction of nitrate (NO₃⁻) to nitrite (NO2⁻). According to Campbell (1988) three forms of NR are known to exist as - NADH-NR with pH optimum of 7.5, NADPH-NR with pH optimum of 6.5 to 6.2 and both NADH and NADPH utilizing forms of NR as electron donars with a pH optimum of 6.5.

The NADH-NR of both monocots and dicots is a dimeric enzyme composed of two identical subunits (homodimer). Each subunit consists of one flavine adenine dinucleotide (FAD), one cytochrome-b (cyt b 557) and cofactor containing Molybdenum (Mo.Co) (Campbell and Remmler, 1986 and Campbell, 1988). Campbell (1988) proposed minielectron transport chain as the model of NR Fig.15.

$$NO_3^- + NADH + H^+ \xrightarrow{Nitrate Reductase} NO_2^- + NAD_+ + H_2O$$

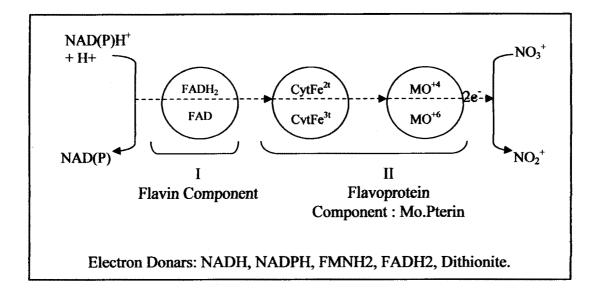
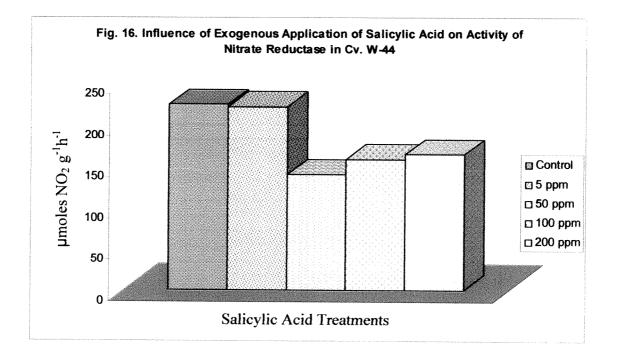
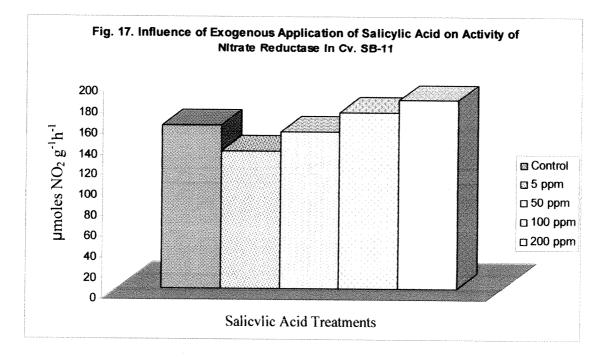


Fig. 15: Minielectron transport chain/Multiple redox enzyme (Campbell, 1988)

Guerrao *et al.* (1981) noticed that during the nitrate reduction, electrons are directly transformed from molybdenum to nitrite. Mostly the NADH functions as an electron donar, electron transport occurs through FAD, Cyt.b 557 and Mo.Co. to nitrate and finally nitrate reduced to nitrite.

NR is the rate limiting enzyme in nitrogen (N) assimilation and is a key point of metabolic regulation (Morilla et al., 1993). NR activity is generally associated with protein synthesis and plant growth, which are affected by abiotic stresses (Sinha and Nicholas, 1981). The various environmental factors including nitrate supply and light intensity regulates the NR activity (Beevers 1969, Srivastata, 1980). The phytochrome also controls the NR activity (Appenroth et al., 1992, Chandok and Sopory, 1996). Circadian rhythms also influence NR activity, protein abundance and mRNA levels (Hoff et al., 1994). Internal factors such as covalent and non-covalent modifications. (Lillo et al., 1996, 1997) and polyamines influences the NR activity (Jain et al., 1997). Solomonson and Barber (1990) have been recognized that NRA is regulated by enzyme modulation and at the level of protein synthesis and degradation. NR is modulated by reversible protein phosphorylation/dephosphorylation (Huber et al., 1993, Kaiser and Huber, 1994). The studies made by several workers suggest the role of phytoharmones in the regulation of NR activity (Campbell, 1999, Pike et al., 2002, Jain, V. and Abrol, 2005). Harmones acts at the level of *de novo* synthesis of the enzyme (Lu *et al.*, 1990, 1992 a and b).





Sengupta and Sharma (1986) investigated the interaction between plant growth regulators and nitrogen metabolizing enzymes in groundnut. It has been reported that in legume plants like groundnut NR enzyme plays an important role in nitrogen metabolism (Chavan, 1987). Phytohormones and synthetic compounds found to influence plant growth and development in groundnut crop. Exogenous application of IAA, GA and kinetin at different growth stages results to rise in the activity of nitrate reductase and biomass. The significant increase in nitrate reductase was important as it is the key enzyme for nitrate reduction in higher plants (Sinha and Nicholas, 1981). Lips and Roth (1969) have been found that GA in combination with cytokinin caused increase in NRA in tobacco plant. ABA enhanced NRA in *Phaseolus aconitifolium* (Sankhala and Huber, 1975). CCC and kinetin pretreated groundnut plants under stress condition enhanced the NR activity (Kutwal, 1989).

Kaur (1987) reported that phenolic compounds caused induction of NRA in two groundnut cultivars. Similarly treatments of phenolic compounds and mixitol found to increase the accumulation of nitrogen in groundnut pods (Grewal, 1989). Mishra et al. (1995) found increased NR activity in leaves of Brassica compestris, treated with polyamines, spermine, spermidine, putresine and cadavarine. Phenolic compounds like SA, gallic acid, resorcinol and tannic acid accelerates the nitrate reductase activity in three cultivars of soybean (Kumar and Mani, 1999). Rane and his coworkers (1995) suggested that SA has a protective role on NRA in wheat leaves. Foliar application of SA after 12, 24, 36 days enhanced NRA in soyabean (Kumar et al., 1998). According to Asthana and Srivastava (1978) presowing soaking treatments with asorbic acid and asorbic acid in combination with SA enhanced NR activity in maize seedlings. Jain and Srivastava (1981) have been found to increase in NRA in roots of maize seedlings by SA treatments. Jaleel et al. (1998) recorded induction in NRA by lower concentration of SA in Vigna mungo. The higher doses (above 100 ppm) of SA caused stimulation of NRA in germinating mung seeds (Mohariker, 2001). Salicylic acid, brassinosteroids, triacontanol found to increase in NRA and uptake of nitrogen in Pearl millet (Sivakumar et al., 2002). Similarly NRA was increased especially by 100 ppm foliar application of SA in Lycopersicon esculentum L. (Kalarani et al., 2002 a). They also noticed similar results in Pearl millet (2000 b). Singh and Usha (2003) have reported that SA protected and maintained NR activity under water strees in wheat. SA caused induction in NR acivity in Brassica juncea (Fariduddin et al., 2003).

In the present investigation the activity of NR was measured in leaves because Chavan (1987) noticed higher NRA in leaves as compaired to stem and root. The SA application caused the stimulation of NR activity in Cv. SB-11 by higher concentration of SA. However, the NRA was decreased significantly with 50 ppm SA treatment in Cv. W-44. These findings indicate a key role of application of SA in nitrogen assimilation before flowering of groundnut for the fulfillment of nitrogen requirement in groundnut Cv. SB-11 than Cv. W-44.

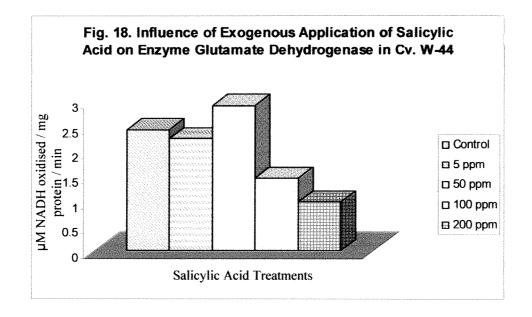
i) GLUTAMATE DEHYDROGENASE

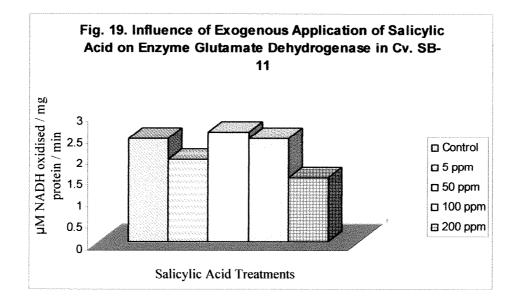
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The effect of foliar application of salicylic acid on the behavior of enzyme dehydrogenase in the groundnut cultivars, W-44 and SB-11 is recorded in Fig. (18-19). Activity of enzyme is noticeably increased by 50 ppm SA treatments in both the groundnut cultivars. However, other SA concentrations inhibited the activity. Especially 200 ppm SA treatment caused significant decrease in both cultivars of groundnut.

Dehydrogenases play an important role in cellular metabolism. Dehydrogenases are involved in physiological processes like glycolysis, pentose phosphate pathway and TCA cycle in seeds (Chakravarti and Burma, 1959). Earlier Price and Thimann (1954) has been established the relationship between dehydrogenase activity and respiratory rate. Dehydrogenases are oxidising enzymes catalyzing electron transfer from the donor to an acceptor other than molecular oxygen. The cytosolic dehydrogenase generate reducing potentials, NADH and NADPH which are involved in various metabolic processes in growing tissues and replenish the mitochondrial compartment with reducing powers in the event of metabolic limitations (Chen *et al.*, 1988). The enzyme dehydrogenase is one of the fundamental energy yielding enzymes in cell metabolism and any disturbance in activity would result in the disruption and alteration of cell growth (Weimberg, 1970 b). Dehydrogenases are also involved in amino acid metabolism.

Glutamate dehydrogenase (GDH) is a ubiquitous enzyme that catalyzes the reversible amination of 2- oxoglutarate to glutamine it results in ammonia utilization or assimilation. It also catalyzes the reverse reaction oxidative deamination of glutamate, this reaction serves as a link between amino acid degradation and the kerbs cycle. The occurance of enzyme has been reported in seeds (Thurman *et al.*, 1965), hypocotyls





(Yue, 1969), roots and shoots of higher plants (Pahlich and Joy, 1971) Glutamate dehydrogenase catalyzes the following reaction,

 $2 \operatorname{Oxo-G} + \operatorname{NADH} + \operatorname{H}^+ + \operatorname{NH}_4^+ \longrightarrow \operatorname{Glutamate} + \operatorname{NAD}^+ + \operatorname{H}_2 \operatorname{O}.$

The enzyme catalyzing the reverse catabolic reaction utilizes NAD⁺, whereas the one catalyzing the forword anabolic reaction can utilize both NADH and NADPH as electron donors. Ca^{+2} acts as an activator for the forword reductive amination, Zn^{+2} and Mn^{+2} can also activate the enzymatic reaction (Chou and Splittsloesser, 1972). Structurally, the enzyme is a homoligomer consisting of six identical subunits of molecular weights 46 to 58.5 D. each (Miflin and Lea, 1982). Schied *et al.* (1980), isolated tetramer of GDH'S from *Pisum* and *Lemna*. Watanabe (2007) has been found 7 GDH isoforms with increases in amination as well as deamination activity.

Recently Watanabe et al. (2007) has been documented that salicylic acid, jasmonic acid and oligosaccharides (OGS) changed the isoenzyme profiles of glutamate degydrogenase (GDH) which may reflect the redox state of Brassica leaves under oxidative stress. Moharikar (2001) has reported that that salicylic acid treatments reduced the dehydrogenase activity in germinating wheat seeds and moong seeds.In contrast to it, Kumar and Mani (1999) have noticed that the foliar application of phenolic compounds such as salicylic acid (SA), gallic acid (GA), resorcinol (Res) and tannic acid (TA) increased the activity of enzyme dehydrogenase in three soyabean varieties (VL Soya 2, Bragg and Shivalik). Thulke and and Conrath (1998) has reported that mannitol dehydrogenase and anionic peroxidase encoding genes are directly responsive to salicylic acid it confirms the role of salicylic acid in activation of defenserelated genes. Recently Martinez et al. (2007) Glutathione dependent formaldehyde dehydrogenase (FALDH) play's an important role in formaldehyde metabolism, defense mechainsm and phytroemediation. They further studied the importance of FALDH in S- nitrosothiols (SNOS), Nitric oxide (NO) related metabolites, which plays a role in signal transduction and defense mechanism (Wendehenne et al., 2002). The genes related to plant hormones involved in the signal transduction pathway and mechanical wounding are down regulated by wounding in JA - dependent pathway and is also transcriptionally activated by salicylic acid (Delledonne et al., 1998, Martinez et. al., 2007) Similarly Diaz et al. (2003) reported that gene encoding FALDH is down

regulated by wounding and activated by salicylic acid in *Arabidopsis* whereas in tobacco salicylic acid enhanced the levels of FALDH and enzymatic activity.

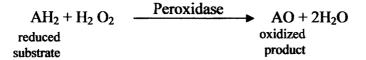
The suppression of dehydrogenase activity due to lower (5 ppm) and higher concentration of SA (100 and 200 ppm) causes reduction in dehydrogenation process. However stimulation of enzyme dehydrogenase activity by 50 ppm SA treatment in both groundnut cultivars reflected positive influence on respiratory process for generating reducing potential required for various metabolic activities of crop.

C) OXIDATIVE ENZYMES

i) ENZYME PEROXIDASE

The influence of various concentrations of SA on the behaviour of enzyme peroxidase in studied groundnut cultivars (SB-11 and W-44) is depicted in fig.20-21. It is evident from the figures that the increase in SA concentrations increased the activity of peroxidase in both groundnut cultivars. The maximum enzyme activity is noticed with 200 ppm SA caused reduction in activity in both the groundnut cultivars.

Peroxidase is a heme containing enzyme and ubiqutosly found in plants. (Welinder, 1992) The prim role of peroxidase is breakdown of $H_2 O_2$ (Cakman *et al.*, 1993).



Besides the degradation of H_2O_2 peroxidase catalyzes the oxidation of NADPH₂, indol acetic acid, phenylpyruvate under aerobic condition without the application of external peroxide (Fric, 1996). Peroxidase in higher plants is glycoproteins and calcium proteins (Welinder, 1985). Heme synthesis of peroxidase occurs in the mitochondria (Chibbar and Van Huystee, 1986). Peroxidase becomes inactive in absence of heme molecule (Chibbar *et al.*, 1984). The structure of peroxidase has been analysed in *Arachis hypogaea* in great detail (Schuller *et al.*, 1996). Heme peroxidases belonging to a superfamily having three classes on the basis of amino acid sequence. Class I enzymes made up of intracellular peroxidases from mitochondria, chloroplasts and bacteria includes cytochrome c peroxidase and Lignin peroxidase (Lip) and class III peroxidases, a plant-specific oxidoreductase

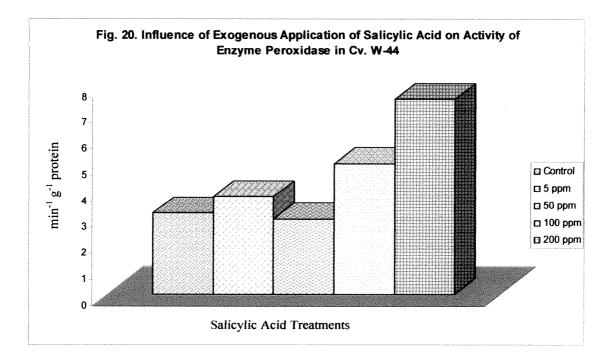
including secretory plant peroxidases (Lopez Molina et al., 2003) and is considered as "Classical" plant peroxidases (Welinder, 1992). Class III peroxidases induced by inducing peroxidase genes via different signal transduction pathways (Hiraga et al., 2001). Dunford (1999) showed that class III peroxidases oxidises a organic and inorganic secretary plant peroxidases. It has been reported that the guiacol peroxidase and ascorbate peroxidase breakdowns the H₂O₂ to H₂O (Asada, 1996, Franck, 1995). According to Nocter and Foyer (1988) enzyme ascorbate peroxidase (APX) scavenges H₂O₂ to water by using assorbate (as the electron donar), the resulted dehydro-assorbate converts to ascorbate by reduced glutathione (GSH-as electron donar) and finally oxidised glutathione (GSSG) is recycled to GSH by NAD (P)H- dependent GR through the ascorbate - glutathione cycle. RAA (1973) has been suggested the histone like function of peroxidase isozymes. Peroxidase present in nucleus found to be involved in the chromosomal organization. Ivanova et al. (1967) investigated the association of proxidase with electron transfer from NADH₂ to cytochrome in mitochondria. The characteristic activity of peroxidase is one electron oxidation (Colona et al., 1999). Peroxidase participated in various physiological processes such as lignification (Ros Barcelo et al., 1989, Chittoor et al., 1999), Suberization (Kollatukudy et al., 1989), auxin catabolism, wound healing and defense mechanism (Breda et al., 1993). Peroxidases carried out the oxidative decarboxylation of amino acids like serine, alanine, phenylalnine, tryptophan and methonine. (Mazelis and Ingraham, 1962) It has been reported the role of peroxidase in the ethylene biosynthesis from ketomethylthiobutric acid or B- methyl thiopropionaldehyde (Ku et al., 1970). Stuhmann and Demorest (1972) showed the involvement of ribosomal peroxidase in the formation of new ribosomes. Fernandes et al. (2006) revealed that anionic peroxidase involved in defense mechanism against pathogen attack.

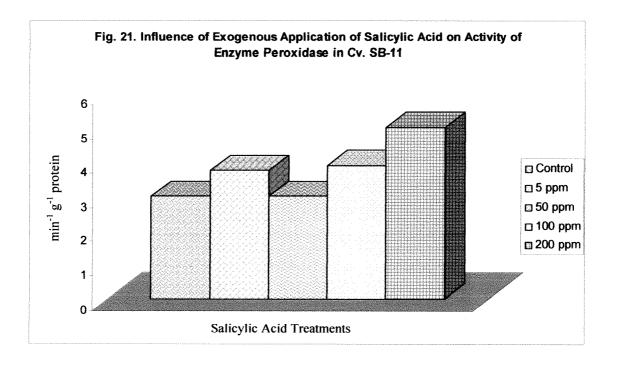
Blee *et al.* (2001) suggested that peroxidase participated in the lignification and defense response against pathogen attack. The role of peroxidase has also been reported in heat stress- tolerance (Dat *et al.*, 1998, Chaitanya, *et al.*, 2002).

Ascorbic acid has been found to be inhibitor of peroxidase influenzing the growth of plant cells (Castillo and Greppin, 1988). The cell wall pH, Ca₂ ions and calmodulin influenced the activity of enzyme peroxidase (Ros Barcelo *et al.*, 1989). The peroxidases occur as isoenzymes in plant species and each isoenzyme containing variable amino acid sequences.

It has been found that salicylic acid and 5- Chloro- salicylic acid supplimented to cucumber seedlings enhanced activity of peroxidase and chitinase enzymes (Siegrist *et al.* 1994). Stitcher *et al.* (1997) found that salicylic acid induced the peroxidase activity which was involved in lignification of cell wall causing an increased resistance to degradation by enzyme secreted resistance to degradation by enzyme secreted resistance to degradation by enzyme secreted by pathogens. According to Zhang *et al.* (2003), the treatment with 0.5 mMol/ L of salicylic acid inhibited the ascorbate peroxidase activity and increased proxidase activity in contrast to it pretreatments of salicylic acid increased the ascobate peroxidase (APX) and inhibited the peroxidase activity in banana seedlings. Foliar application 1.0 mM salicylic acid significantly reduced the paraquat induced retardation of ascorbate peroxidase (APX) and guiacol peroxidase (GPX) and improved the tolerance in *Arabidopsis thaliana* against paraquat induced oxidative damage (Kim *et al.*, 2003). Ananieva *et al.* (2004) found in the barley leaves (*Hordeum vulgare*) that salicylic acid induced the antioxidant enzymes including peroxidase. SA pretreatment increase peroxidase activity in salinity stressed seeding of barley (E1 – Tayeb, 2005).

Fernandes et al. (2006) have recorded two isoperoxidases in cowpea (Vigna unguiculata) leaves. The primary leaves of cowpea treated with 10 mM salicylic acid increased anionic peroxidase activity. They further suggested that anionic peroxidase might be involved in the defense mechanism against pathogen attack. The exogenous application of salicylic acid, brassinolide, chitosan and spermidine improved the activity of peroxidase in cucumber under 200 mmol. L-1 stress (Zhang et al., 2006). Faheed and Mahamoud (2006) found that low concentration of salicylic acid and kinetin enhanced the activity of peroxidase in bean (Phaseolus vulgaris) plants inoculated with TNV (Tobacco Necrosis virus). Similar results were recorded earlier by Clarke et al. (2002 b) in some plant - virus interactions viz. tobacco and TNV, Cucumber and cucumber mosaic virus. Tasgin et al. (2006) studied the influence of salicylic acid on apoplastic enzyme activities. They reported increased activity of apoplastic peroxidase in the leaves of wheat plants. The infection of Zucchini Yellow Mosaic Virus (ZYMV) as well as salicylic acid treatments showed induction in the activity of enzyme peroxidase in the leaves of pumkin (Cucurbita pepo Cv. Eskandarani) indicated that salicylic acid involved in the induction of defense mechanism against ZYMV infection (Radwan et al. 2007) whereas Kang et al. (2003 a, b) suggested that salicylic acid caused stimulation of APX and GPX and both may play key role in removal of H₂O₂. The activity of peroxidase in leaves of two cultivars of





groundnut during different stages of growth was observed by Rane (1987). She noticed that peroxidase activity was not uniform. The stimulation of enzyme upto 50 days followed by sharp decline upto 80 days which further gradually increases upto 120 days.

Earlier Janda *et al.* (2000) found similar results in maize by the treatments of aspirin and benzoic acid concluding the role of SA in improving chilling tolerance (2°C stress.) Mishra and Choudhari (2001) also reported reduction in activity of peroxidase in rice in response to salicylic acid. Larkindale and Huang (2004) recorded that salicylic acid exerted no any effect on the activity of peroxidase in *Agrostis stolonifera*. Tuna *et al.* (2007) reported that maize plants pretreated with salicylic acid caused a decrease in peroxidase enzyme.

In the present investigation increase in peroxidase activity due to higher concentration (200 ppm) of SA treatment in both the groundnut cultivars is significant. This is in agreement with several earlier reports. Similarly non-occurrence of any pathogen on both cultivars is also in agreement with enhanced activity of enzyme peroxidase (Blee *et al.*, 2001). However, in view of various roles of peroxidase it is difficult to point out the exact reason for stimulation of peroxidase activity. But as groundnut is C_3 plant obviously occurrence of photorespiration may be one of the cause for stimulation of enzyme activity.

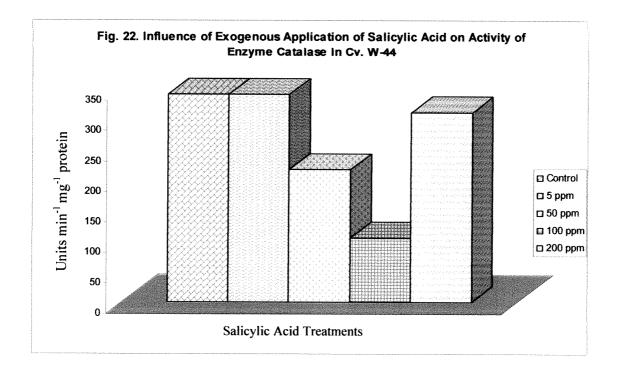
ii) Enzyme Catalase

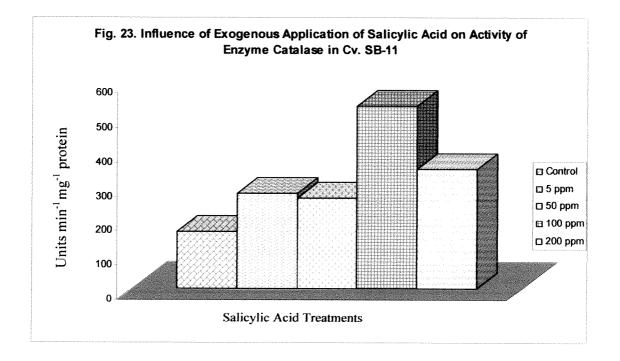
The Fig 22 and 23 indicates the effect of foliar application of salicylic acid on activity of enzyme catalase in groundnut Cv. W-44 and SB -11 respectively. In case of Cv. W-44 the enzyme activity is declined in salicylic acid treated plants over control. However the activity of enzyme catalase is increased by salicylic acid treatments in Cv. SB- 11. It is more significantly increased by 100 ppm Salicylic acid.

Catalase is a common enzyme involved in decomposition of hydrogen peroxide. Hydrogen peroxide (H₂O₂) is a harmful by product, to avoid metabolic damage it must be immediately converted into less toxic substances. Catalase rapidly catalyzes the decomposition of H₂O₂ into less reactive gaseous oxygen and water molecules. The plants exposed to environmental stresses such as excess excitation, drought and cold causes promotion of H₂O₂ generation (Bartosoz, 1997). Catalase is generally located in a cellular organelle; the peroxisome (Albert *et al.*, 2002). One molecule of catalase can convert millions of hydrogen peroxide molecules to H₂O and O₂ per second. Catalase is actively involved in photorespiration and symbiotic nitrogen fixation, it also produced during β -oxidation. In pathogen infected plants catalase functions as a potent antimicrobial agent. Excess superoxide results in the synthesis of hydroxyl radicals and hydrogen peroxide by several reactions (Winterbroun, 1981, Szigeti *et al.*, 2001). Hydrogen peroxide is a form of reactive oxidative species (ROS) and its synthesis brings about by enzyme superoxide dismutase in uncontrollable manner during the oxidative as well as photosynthetic electron transfer processes.

According to Neill *et al.* (2002) biotic and abiotic stresses, such as high temperatures; UV radiations, Ozone exposure, phytohormones like ABA, dehydration, wounding, and pathogenesis promoted H_2O_2 generation. A biotic and abiotic stresses also induces active oxygen species scavenging enzymes including catalase (Chaitanya *et al.* 2002). H_2O_2 can serves as a signalling molecule in plants under stress conditions (Dat *et al.* 2002). H_2O_2 at physiological concentrations are non – toxic but their metal ion dependent conversion into hydroxyl radicals causes mutation of DNA, lipid peroxidation and denaturation of proteins (Bowler *et al.*, 1992, Foyer *et al.*, 1994). It has been reported by Willekens *et al.* (1997) that catalase can serve as a sink for H_2O_2 . It is emphasized by Kremer (1970) that besides H_2O_2 , enzyme catalase oxidises other substances. The catalase mitigates the cellular damage by converting superoxide anion to H_2O_2 and H_2O_2 to H_2O (Scan - dalios, 1993).

The decline in catalase activity with increasing maturation period was investigated by Rane (1987) in two cultivars of groundnut. Several reports are available emphasizing the role of SA in regulating the activities of antioxidant enzymes (Ma *et al.*, 1998, He *et al.*, 2002). Du and Klessing (1997) reported the requirement of SA for the activation of defense responses in catalase- deficient tobacco plants. Several workers have found SA-binding protein in the leaves of tobacco and identified it as catalase (Du and Klessing, 1997, Chen and Klessing, 1991, Chen *et al.*, 1993 a and b). Ananieva *et al.* (2002) found enhancement in photorespiration which is regarded as a protective alternative pathway against photoinhibition and photoxidation in chloroplast. SA pretreatments found to be reduced the catalase activity in *Arabidopsis* plants (Kim *et al.*, 2003). Shim *et al.* (2003) reported the correlation between inhibition of catalase activity and increase in endogenous salicylic acid in plants. SA induced H₂O₂ detoxyfying enzymes including catalase (20%), Superoxide dismutase (17%) and peroxidase (25%) in barley plants under paraquat stress and enhanced stress tolerance





(Ananieva *et al.*, 2004). In supporting to these results earlier Ananieva *et al.* (2002, 2004) have also shown that SA treatment enhanced the activity of catalase, peroxidase and superoxide dismultase (SOD) in choroplast as well as other cellular compartments (cytosol, peroxisome) leading to recovery of chloroplast. Foliar application of SA increases the catalase activity with the removal of H_2O_2 however foliar treatment along with addition of SA (1mm) with nutrient solution showed decreased activity of catalase in *Cucumis sativa* (Shi *et al.*, 2006). Sahel *et al.* (2007) observed that SA application caused induction of catalase activity leading to protection from heat stress injury in moong bean plants. Zhang *et al.* (2006) found that root injection and foliar spray of SA, brassinolide, chitosan and spermidine increased the activity of catalase in cucumber seedlings under salt stress. Lower and higher foliar H_2O_2 content correlated to low and high SA levels (Alfonso *et al.*, 2006). As the catalase is a H_2O_2 degrading enzyme SA treatment caused reduction in CAT activity and induction of endogenous H_2O_2 levels in maize plants at 25^0 c (Bedi and Dhingra, 2007).

Chen *et al.* (1993 a,b) found that SA inhibited H_2O_2 degrading acitivity of catalase and stimulated the H_2O_2 levels or H_2O_2 derived reactive oxygen species (ROS). Whereas some reports revealed that higher levels of H_2O_2 induced the SA synthesis. These results confirmed that H_2O_2 acts as upstream of SA in signal transduction pathway (Leon *et al.*, 1995, Neuenschwander *et al.*, 1995, Summermatter *et al.*, 1995). Similarly Moharikar (2001) also found reduced catalase activity in germinating seeds of wheat. SA reduced the catalase activity in roots and shoots of rice (Mishra and Choudhary, 2001). He *et al.* (2001) studied the effect of SA on antioxidant enzyme activites in heat stressed Kentucky bluegrass. They found that SA treatment reduced the levels of H_2O_2 at 2 and 12 h. catalase enzyme significantly at 12 h and activity of heat stress. It has been reported that application of SA, CaCl₂ and H_2O_2 reduced the activity of calalase prior and after 48 hrs of heat stress in *Agrostis stolonifera* (Larkindale and Huang, 2004).

In the present investigation the behaviour of enzyme catalase is not uniform. Both groundnut cultivars show different responses for the enzyme activity in response to different SA treatment. In the Cv. SB-11 stimulation of enzyme catalase activity indicates activation of antioxidant defense mechanism.

4. STOMATAL CONDUCTANCE AND WATER RELATIONS:

The qulitative determination of influence of foliar application of salicylic acid on the rate of stomatal transpiration and moisture content in leaves, shoot and root is depicted in Table No.5 and 6 respectively. Salicylic acid controlled the transpiration rate with 100 and 200 ppm SA spray in Cv. W-44 while opposite trend is noticed in Cv. SB-11. In Cv. W-44, application of SA is responsible for retaining maximum moisture content in leaves in shoot significant results are obtained with 5 and 50 ppm SA but reduction in moisture content was observed in roots. Incase of Cv. SB-11, SA reduced the moisture content in leaves while moisture was retained in shoot and roots by all SA treatments except 100 ppm SA concentration.

Transpiration is a loss of water in the form of water vapours from the aerial plant parts. It is a vital process controlled and regulated by the cells in the plant. The leaf epidermis is considered as multiperforated septum, through its pores outward diffusion of water vapour occurs. Besides the diffusion of water vapours gases like carbon dioxide, oxygen, trace amounts of other volatile substances exchanged between higher plants and the external atmosphere. In higher plants stomatal pores are the mean of gaseous exchange.

The factors like shape and size of substomatal cavity, stomatal pore and size of stomatal pore influences the stomatal resistance. The stomatal pore size is considered as most important factor. The smaller the stomatal openings, the greater the resistance to outward diffusion of water vapours. The turgidity of guard cells leads the changes in stomatal pore size. Malate ions and K^+ also influences the opening and closing of stomata. The loss of excess water through stomata results in reduction in photosynthetic rates and other metabolic processes.

Earlier the role of salicylic acid in stomatal functioning has been emphasized by Larque-Saavedra (1979), Rai *et al.* (1986) and Aldesuquy *et al.* (1998). Acetyl Salicylic acid functions as antitranspirant in leaves of *Phaseolus vulgaris* (Larque-saavedra, 1978). Larque-Saavedra (1979) noticed closure of stomata within 13 min with a treatment of 10 mM acetyl-salicylic acid (ASA) solution in *Commelina communis*. Manthe *et al.* (1992) studied the effect of salicylic acid on the growth and stomatal movements of *Vicia faba* L. They reported that SA concentration higher than 3.5 mM only affected the stomatal pore width whereas lower concentration of SA as 0.001 mM leading higher sensitivity of guard cells in epidermal peels resulting in stomatal closure.

Salicylic Acid	Stomatal conductance			
concentration (ppm)	W-44	SB-11		
Control	+++	++		
5	++	+		
50	++	++		
100	+	+		
200	+	+++		

Table 5 : Effect of foliar application of Salicylic Acid on Stomatal transpiration.

* '+' sign indicate intensity of pink colour.

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Cultivar	Salicylic Acid concentration (ppm)	Moisture content (%)		
		Leaves	Shoot	Root
W-44	Control	74.35	67.44	63.20
	5	77.77	69.96	56.03
	50	77.63	68.98	61.11
	100	73.97	68.75	60.22
	200	80.48	68.92	61.06
SB-11	Control	93.33	72.26	61.78
	5	81.88	75.55	66.84
	50	85.71	74.37	66.67
	100	78.48	71.66	53.14
	200	76.67	73.61	63.89

-

Table 6: Effect of foliar application of salicylic Acid on the Moisture content,

Lower concentrations of Salicylic acid decreased stomatal aperatures resulting reduced transpiration in soyabean plants (Barkosky and Einhelling, 1993).

Mori *et al.* (2001) recorded induced stomatal closure in SA treated *Vicia faba*. Salicylic acid, acetyl-Salicylic acid and gentisic acid controls the opening of stomata in corn and soyabean (Khan *et al.*, 2003). Mateo (2006) noticed rapid stomatal closure in *Arabidopsis* leaves under short-day conditions and inhibition of photosynthetic electron transport under low light conditions with SA application.

The recent investigation by Stevens and Senaratna (2006) reported that SA causes the higher transpiration rates and stomatal conductance in saline stressed tomato plants. Tuna *et al.* (2007) found that salicylic acid treatments increased relative water content (RWC) in salinity stressed maize plants. These results correlated with the decreased transpiration rates by salicylic acid and its derivatives putforth by Aktas (2001) in *Vitis vinifera*.

In the present investigation, the poor intensity of pink colour indicates lowering of transpiration rate by closing of stomatal aperture with higher concentration of SA (200 ppm) and it is significant in Cv. W-44, than Cv. SB-11. However, in case of Cv. SB-11 the lower concentration of SA (5 ppm) significantly reduced transpiration rate is in agreement with our findings of effect of SA on K+ status as well as recall the findings of Manthe *et al.* (1992).

Similarly it is evident from our findings that the moisture content in the leaves of both the cultivars correlated with these results of stomatal conductance.

5. INORGANIC CONSTITUENTS

The several workers has been studied the requirements of nutrients of groundnut crop (Singh, 1999; Singh *et. al.*, 1995, Singh and Chaudhari, 2007). Insufficient mineral supply causes nutritional disorders in groundnut (Beringer and Taha, 1976). The literature of influence of SA on mineral status in plants is very scanty. In present work the influence of different concentrations (5, 50, 100 and 200 ppm) of salicylic acid on uptake of different mineral ions in groundnut cultivars, SB-11 and W-44 is depicted in fig. (24-31).

a) PHOSPHORUS

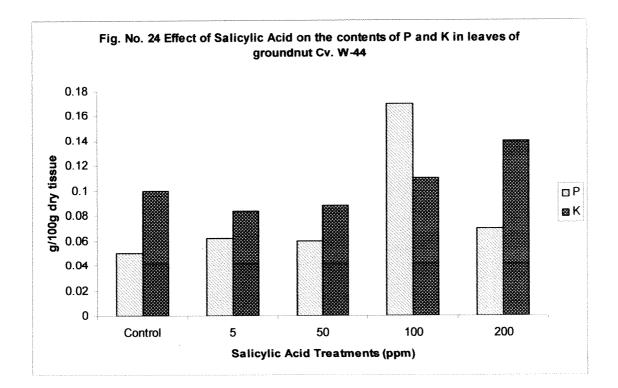
The content of phosphorus is increased with increasing SA concentrations, in both the studied groundnut cultivars (fig.24-25). The phosphorus content goes on

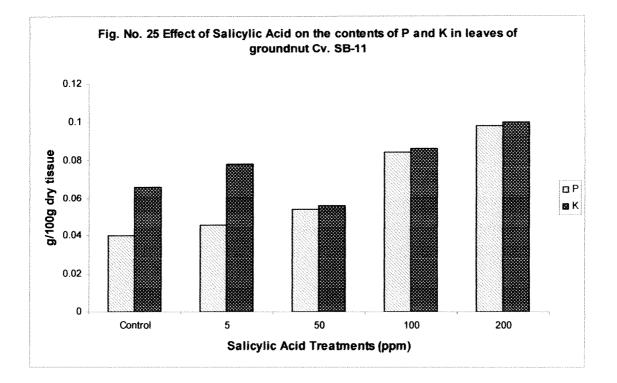
increasing in both cultivars with all concentrations of SA treatment. But in Cv. SB-11 'P' accumulations enhanced successively.

Phosphorus plays very significant structural role in nucleic acids, nucleotides and phospholipids. Besides structural role phosphorus has an important function in intermediately metabolism. It is an important constituent of high energy component, ATP and the coenzymes, NADP involved in the process of respiration, photosynthesis, nitrogen metabolism, carbohydrate metabolism and fatty acid synthesis (Marschner, 1986). Phosphorus is the part and parcel of RuBP and PEP, the main CO₂ acceptors in plants. It is a component of various sugar phosphates of Calvin cycle, glycolysis and pentose phosphate pathway. It is a major component of many metabolically important molecules such as sugar phosphates, nucleotides, nucleic acids, phospholipids and coenzymes (Marschner, 2002).

Phosphorus plays vital role in growth and development of groundnut. Phosphorus starvation has rapid influence on the mineral nutrition of groundnut. Basha and Rao (1980) have noticed the effect of 'P' and 'K' deficiency on CO₂ fixation and translocation in groundnut plants. Its deficiency is evident when number of pods produces one kernel (Dwivedi, 1986). Rane (1987) recorded the changes in 'p' content in the first leaf of groundnut, at different stages of growth. She observed that 'P' content increased upto 40 days but slight decline therafter may helps to maintain optimum 'P' status in plant. In groundnut, P is very critical at flowering and pod formation stages (Singh et al., 1991). Application of P increased the nodulation, N2fixation and N contents of the kernel and foliage (Singh, 1996a). According to Singh (1999) deficiency of P in groundnut causes purpling of leaf margin and stunted growth. The deficiency spreads from older leaves to other leaves. Phosphorus enhanced root formation (Reddy et al., 1981). The directly and indirectly involvement of P and N in the production and enlargement of new cells and tissues caused increase in shoot length which are responsible for better exposure of leaves for solar energy harvesting and for larger leaf area have been noticed by Siddqui et al. (2007). These findings correlated with our investigation with respect to increase in fresh weight, dry weight and leaf area in SA treated groundnut cultivars. Anuradha et al. (1995) reported significant decrease in shoot-root ratio in 'P' deficient groundnut plants.

Alsaadawi *et al.* (1986) has been reported that ferulic acid, caffeic acid, proto-catechuic acid and syringic acid negatively influence phosphorus uptake in soyabean and cowpea seedlings. Naik *et al.* (2005) reported induction in 'P' content in groundnut on





application of NAA, KNO₃, FeSO₄, ZnSO₄, and MgSO₄. Tillberg (1970) has been reported that SA decreases the total inorganic phosphate uptake in *Scenedesmus*. It was revealed that increased phosphorus content increases N_2 -fixation and nitrogen content of kernel and foliage. Salicylic acid pretreatments found to be increased the phosphorus content in barley grains (El- Tayeb 2005).

Thus our results revealed that increased phosphorus by SA treatment might be involved in metabolism especially regulation of photosynthesis and N₂-fixation in groundnut.

b) POTASSIUM

The uptake of potassium by different concentrations of SA in Cv. W-44 and SB-11 is depicted in fig (24-25).

Both the studied cultivars of groundnut performs similar response to SA treatments. The higher concentrations of SA (100 and 200 ppm) significantly increased the potassium level; however the potassium accumulation is reduced by 50 ppm SA treatment.

After phosphorus and nitrogen, potassium is most important mineral necessary for normal plant growth and metabolism. Potassium does not appear as a constituent of any plant metabolite and it is not incorporated in plant structure, thus we do not have clear idea about its mode of action. Potassium is an inorganic osmoticum in number of plants (Cram, 1976; Ortiz *et al.*, 1994). It is an activator in protein metabolism. It is necessary for formation of sugars, Starch, Carbohydrates, cell division, cell extension and storage of assimilates (Lindhauer, 1989). It also helps to adjust water balance, stomatal movment, improves stem rigidity and cold hardiness as well as increases the oil content of fruits. Potassium is taking part in metabolic processes such as photosynthesis, and respiration because it acts as a counter ion for H⁺ ion flux across the thylakoid membrane of chloroplast and mitochondrial membranes (Pfleger, 1972). It was observed that, potassium acts as an activator of more than 50 enzymes such as pyruvate kinase, Phosphofructokinase, glutathione synthatase, aldolase, RuBP carboxylase (Peoples and Koch, 1979) and nitrate reductase (Gutierrez *et.al.*, 1998).

Potassium is essential for growth and development of groundnut. It was found that 'K' deficiency influenced the rate of translocation (Basha and Rao, 1980). Its deficiency in groundnut causes yellowing of the margins of old leaves interveinal chlorosis, necrosis and finally falling of leaves. Its deficiency also appears to reduce root growth and flowers, peg and kernels (Singh, 1999). The normal K content in leaf is 1.5% (Singh, 1999). According to Singh (1996b) higher levels of k absorbed by groundnut and accumulate in leaves, stems and shells but not in seeds.

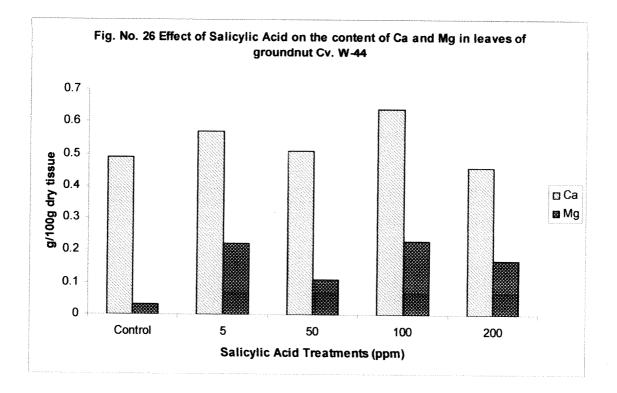
Harper and Balke (1981) reported the inhibition of potassium content in oat roots by SA treatment. Alsaadawi *et al.* (1986) in cowpea plants was noticed reduction in potassium levels under the influence of some allelochemicals.

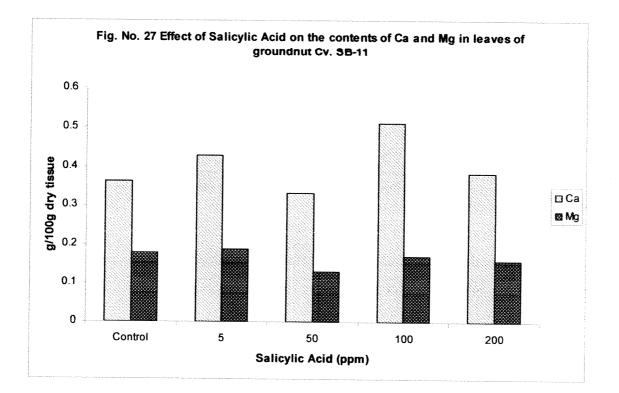
In present investigation stimulation in uptake of potassium due to SA application might be mitigate its deficiency symptoms and controlling transpiration rate by influencing stomatal movements as well as facilitate other metabolic activities in groundnut.

c) CALCIUM

The effect of various concentrations of SA on Ca uptake depicted in Fig. 20-27.In groundnut cultivar, W-44 the calcium uptake increased with increasing SA concentrations and it is significant by 5 and 100 ppm SA treatment. Whereas in SB-11 the results are more significant by 5 and 100 ppm SA, however levels of Ca slightly declined by 50 ppm concentration of SA in both cultivars.

Divalent Ca^{2+} is easily available for plant growth. Calcium is well known for detoxifying higher concentrations of other mineral elements in plants (Marschner, 1986). Calcium activates some enzymes and many are inhibited such as esterase, pectin esterase, lipoxygenase, nucleases, protein kinase, pyruvate kinase, polygalactouronic transaminase, glucose-6-phosphate dehydrogenase (Clarkson and Hanson, 1980). Such inhibitory action of enzymes is necessary for cells to maintain their low concentrations in cytosol where many enzymes exist. Apoplastic and cytosolic calcium plays an important role in regulation of guard cell turgor and stomatal aperture. Calcium also affects chromatin organization, enzyme conformation and regulation of many metabolic processes through calmodulin (Clarkson and Hanson, 1980, Cormier et al., 1982). Calcium deficiency leads to reduce the activities of nitrogenase and nitrate reductase enzymes (Gaudinova, 1983). In plant cell Ca present in association with protein, nucleic acids and lipids to affect several functions of cell membrane, chromatic organization as well as enzyme activities. The all important Ca²⁺ modulated proteins are calmodulin. The Ca^{2+} - calmodulin regulates enzymes like NAD kinase, β -glucan synthase, Ca²⁺ and H⁺-ATPase, NAD⁺-oxiode-reductase and protein kinase, calcium





increases net absorption of potassium (Ortiz *et.al.*, 1994) whereas excess concentration of Ca^{2+} in cytosol leads to cytotoxocity (Wang and Li, 1999, Jiang and Huang, 2001). Calcium is involved in the regulation of plant responces to several environmental stresses (Jiang and Huang, 2001, Nayyar, 2003).

Calcium is found to be abundant in many plants especially legumes. In groundnut calcium is very essential for the gynophores formation and pod setting. Instead of root system, peg and pods of groundnut absorbs calcium (Singh, 1999). Thus calcium content retained in leaves which do not fulfil this calcium requirement (Rane, 1987). Calcium deficiency causes yellowing of leaf margins, development of localized pitted spots on lower surface and large necrotic spots appear on both the leaflets surfaces.Often lack of Ca can reduce the yield and quality more than any other element. Ca deficiency also causes uneven growth of pods and development of 'pops' unfilled pods. It has been reported by Pal and Lalorya (1973) that low calcium levels leads to accumulation of amino acids and amides in roots and leaves of groundnut (). Sudarsnamma and Swammy (1987) studied that calcium in the form of gypsum increased the protein and nucleic acid content in groundnut cultivars JL-24, TMV-2 and Kadiri-3. Whereas calcium also enhanced the activities of enzymes nitrate reductase and glutamate dehydrogenase in groundnut cultivars JL-24 and TMV-2. The application of calcium increased the shelling percentage and oil yield of groundnut (NRCG, 1994).

The literature on the effect of SA on uptake of Ca is not available. But allelochemicals such as benzoic acid and cinnamic acid have been found to increase calcium levels in the soyabean roots. Castro *et al.* (1984) reported that plant growth regulators such as chlormequat, SADH, GA and IAA reduce calcium content in groundnut leaves. Al – Hakim *et al.* (2001) has been found that SA induced the uptake of calcium in salinity stressed wheat plants.

It is evident from the present investigation that Ca content in studied Cv. W-44 and SB-11 increased by the SA treatment and Ca deficiencies like 'pops' is not evident in higher SA concentration treated groundnut plants. Similarly it may fulfil the requirement of this element by both the groundnut cultivars.

d) MAGNESIUM

The effect of various concentrations of SA on uptake of Mg^{+2} is depicted in fig.26-27. Magnesium levels are increased by all SA treatments SA treatments in Cv. W-44, and it is more significant by 5 and 100 ppm SA treatment. However opposite trend was noticed in Cv.SB-11.

Magnesium is a mobile, strongly electropositive, divalent element in plant. It plays prime role in plant metabolism. It is a critical structural component of the chlorophyll molecule which is a basic component of light reactions of photosynthesis. Magnesium is necessary for functioning of plant enzymes to produce carbohydrates, sugars and fats. Several enzymes involved in carbohydrate metabolism require magnesium as an activator in which ATP is involved.

It also acts as an activator for enzymes involved in the nucleic acid synthesis, synthesis of DNA, RNA from nucleotide polyphosphates. It forms a bridge between the phosphate structure of ATP, ADP and the enzyme molecule. Mg functions as bridging element, it binds the subunits of ribosome together and EDTA causes dissociation by its removal of the magnesium ion from the ribosome (Cammarano *et al.*, 1972). It assigns its role in protein synthesis. Mg uptake is influenced by some factors like Mn^{2+} , K^+ , NH₃ and low pH. Thus high concentration of Mg^{2+} and K^+ are essential in the chloroplast and cytoplasm to maintain high pH (6.5 to 7.5). The activity of RuBP carboxylase requires Mg^{+2} and k^{+2} . Mg also increases the activity of enzyme glutamate synthatase (O' Neal and Joy, 1974).

Groundnut plant requires high amount of Mg after 30 days of plant growth (Singh, 1999). The most common symptoms of Mg deficiency in plants is chlorosis of older leaves, chlorosis is followed by anthocynin pigmentation and finally leading to necrosis. Appearance of Tikka disease on groundnut plant is considered as a sign of magnesium deficiency. Plant growth regulators, SADH, increases the magnesium content in groundnut leaves (Castro *et al.*, 1984).

The SA treatment increased the uptake of magnesium in wheat plants grown under salinity stress (Al - Hakim *et. al.*, 2001). Similarly, Alpaslan *et al.* (2005) reported that a SA treatment increases the Magnesium content in maize plant. Our findings are correlated with these investigation as well as increased content of chlorophylls in Cv. W-44 in response to SA treatments. The appearance of Mg deficient-tikka disease is evident in leaves of control groundnut plants is reduced with SA treatments, noticed in Cv. SB-11 (photoplate No. 8) than Cv. W-44.

e) IRON

The effect of SA on uptake of iron is recorded in fig. (28-29), is reduced with the levels of iron enhanced in both cultivars due to 5 ppm SA treatment. However further increase in SA treatment shows differential responces in both cultivars. In Cv. W-44 stimulation of iron content noticed by 5 and 200 SA doses while in Cv. SB-11 the accumulation of iron restricted to only 5 ppm SA treatment. The reduction in Fe uptake is recorded by 50 ppm SA treatment in both groundnut cultivars.

Iron is most important microelement has the greatest biological importance. It is necessary for many enzyme functions as a catalyst for the synthesis of chlorophyll. It is essential for the young growing parts of plants. There are two groups of iron containing proteins, hemoproteins and iron sulphur proteins. (Sandmann and Bogger, 1983) The iron porphyrins (Home protein) i.e. cytochromes are the iron containing prosthetic groups. Cytochrome plays major role in respiration and photosynthesis through electron transfer chain. The iron-sulphur proteins plays vital role in respiration through electron transfer chain. Iron is an important constituent of sulphur protein-ferredoxin which is an indispensable part of photosystems, sulphate reduction and nitrate reduction.

Iron is also constituent of leghaemoglobin which is participated in the mechanism of nitrogen fixation in the leguminous plants. The iron is acts as an activator for the enzymes involved in number of metabolic reactions including catalase, peroxidase and cytochrome oxidase. The enzyme carprophyrinogen oxidase is an iron protein carried out the oxidative decarboxylation of Mg-protoporphyrin. The role of iron is very well documented in the biosynthesis of chlorophyll. About 0.01% concentration of iron is optimum for the proper growth of plant (Stout, 1961). Marschner (1986) has reported that about 80% of the Fe is localized in chloroplast of green leaves. Iron is stored in stroma of plastids in the form of ferric phosphoprotein referred as phytoferritin.

The deficiency of iron causes number of metabolic disorders in plants. The iron deficiency causes appearance of chlorosis and necrosis of young leaves. The occurrence of iron chlorosis is a serious problem in several crops. It is a common in groundnut growing areas. The Fe deficiency in groundnut causes rapid expansion of leaves, interveinal chlorosis and severe deficiency results in white papery leaves (Singh and Dyal, 1992). The iron deficiency limits nodule development in groundnut grown in calcarios soil (O' Hara et al., 1988). NA is a chelate for Fe (II) dependent processes

such as regulation of iron enzymes, phloem loading or unloading of iron and regulation of iron deficiency response mechanism.

Aly and Soliman (1998) found that SA treatment mitigate the iron chlorosis in soyabean grown under calcarious soil.

The foregoing account clears that SA treatment is more favorable to Cv. W-44 than Cv. SB-11 for Fe mediated metabolic processes.

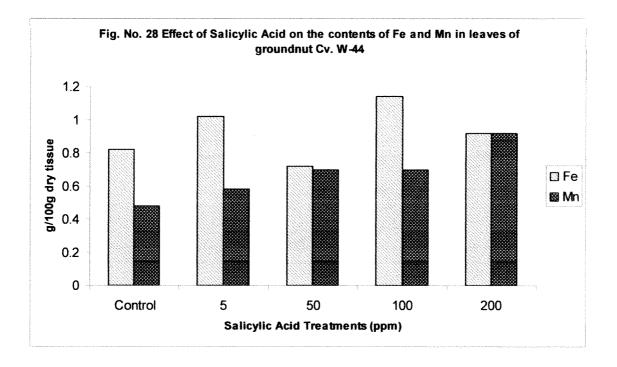
f) MANGANESE

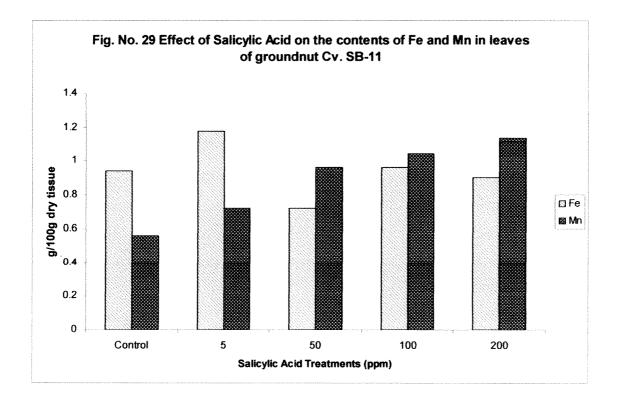
The effect of SA on the accumulation of manganese is recorded in fig. 28-29. The manganese content is enhanced successively with increased SA concentration than control in both the cultivars under investigation. The manganese uptake is found to be more by higher SA treatments (esp. 100 and 200 ppm).

Manganese is one of the most essential macronutrient in plants. Manganese acts as enzyme activator in respiration and nitrogen metabolism. It is considered as an important metal ion of kerbs cycle. It plays a direct role in photosynthesis as it is involved in photoxidation of water during photolysis of water and PS II contain mangoprotein catalyses early stages of oxygen evolution. Mn is involved in the biosynthesis of fatty acids as it is a component of biotin enzyme. It is also related to stability of thylakoid structure. It is involved in the oxidation of IAA, a natural auxin in plants. It can be replaced by other divalent cations such as Mg²⁺, Co²⁺, Zn²⁺ and Fe²⁺ Mn is a structural constituent of ribosome. It activates RNA polymerase, NADP - Malic enzyme, NAD - Malic enzyme and PEP carboxylase. These are the manganese specific enzymes.

The manganese deficiency symptoms may occur first in young leaves and causes chlorosis and necrosis in the interveinal areas of the leaf, and it termed as 'Marsh spots' (Bussler, 1960). According to Stout (1961), 0.005% Mn is sufficient for the growth of plants. Groundnut plant requires about 20 to 40 ppm Mn concentration of leaf tissue, (Reid and Cox, 1973). According to Singh (1994) in groundnut leaf Mn levels ranges from 50-100 ppm and deficiency occurs below 20 ppm.

The results indicate a favorable influence of SA on manganese uptake in studied cultivars of groundnut. Reported range of Mn is correlated with investigated range. The optimum levels of Mn overcome the Marsh spots on leaves.





g) ZINC

The effect of various concentrations of SA on the uptake of zinc is depicted in fig.(30-31). In Cv. W-44 the level of Zn is increased with successive increase in SA concentration whereas in Cv. SB-11 its level is declined initially by 5 and 50 ppm SA but elevated by 100 and 200 ppm SA.

Zinc is a functional, structural and regulatory cofactor of a large number of enzymes including Cu-Zn superoxide dismutase, carbonic unhydrase, proteinase, peptidases, glutamic acid dehydrogenase, isomerases, aldolases, transphosphorylases RNA and DNA polymerases. It is essential to carbohydrate metabolism, protein synthesis and internodal elongation. Zinc acts as an activator of several enzymes, alcoholic dehydrogenase, pyridine nucleotide dehydrogenase and carbonic unhydrase. The first Zn containing enzyme is carbonic anhydrase found in marine plants and animals. It may also acts as an activator for some phosphate transferring enzyme including hyxosekinase, or triose-phosphate dehydrogenase. It may be involved in the biosynthesis of indole-3-acetic acid (IAA), natural auxin. It has been reported marked decrease in auxin content of zinc deficient tomato plants and an increase in IAA content when zinc was added to the deficient plant. It may also play an important role in nitrogen metabolism (Reddy and Rao, 1979). The most prominent symptom of Zn deficiency is an interveinal chlorosis starting at the tip and margins of the older leaves. Chlorosis followed by white necrotic spotting, small leaves, shortened internodes and stunted growth. Zinc deficiency leads to iron deficiency causing similar symptoms.

Deficiency occurs on eroded soils and is least available at pH range of 5.5 - 7.0. Zinc deficiency leads to accumulation of soluble nitrogen compounds such as amino acids and amides. It can thus, confirms the important role of Zinc in protein synthesis. According to Singh (1999) deficiency of Zn in groundnut occurs mainly in the upper leaves with irregular molting and interveinal chlorosis. Severe deficiency causes chlorosis of entire leaf. Dwivedi (1986) found that leaf tissue less than 20 ppm showed Zn deficiency.

Malewar *et al.* (1982) found that increased content of zinc was enhanced the nodulation process in groundnut. Our results are also correlates with this findings.Baziramkenya (1994) reported that the benzoic acid, cinnamic acid causes Zn accumulation in soybean.

The findings of present investigation revealed the favourable influence of higher treatments of SA (esp. 100 and 200 ppm) on accumulation of Zinc. It can thus, assumed

that SA might involve several metabolic activities in groundnut and mitigating zinc deficiencies. As zinc acts as cofactor for SOD, it might also be involved in the defense mechanism of groundnut plants.

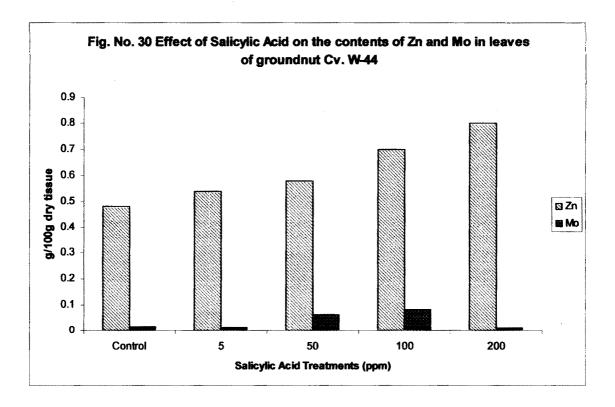
h) MOLYBDENUM

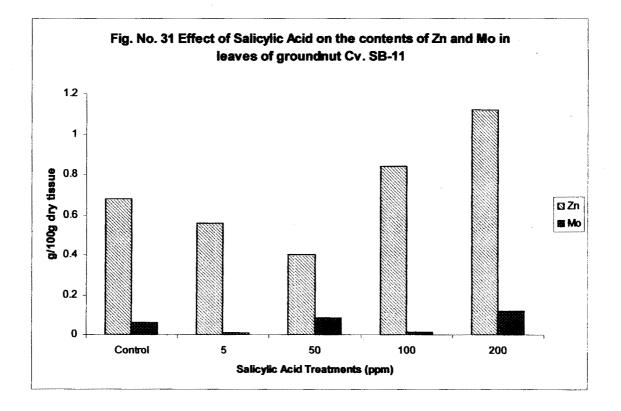
The changes in uptake of Mo due to SA treatments are recorded in fig. 30-31. Molybdenum uptake enhanced with 50 and 100 ppm SA treatments in Cv. W-44. Such stimulatory effect is observed only by 50 and 200 ppm SA in Cv. SB-11.

Molybdenum is considered as an important element for the growth of higher plants (Arnon and Stout, 1939). Molybdenum plays a key role in nitrogen fixation and nitrogen assimilation. Molybdenum is a structural component of the enzyme nitrate reductase that reduces nitrates to ammonia. The nitrogen fixing root nodule bacteria requires molybdenum. In absence of molybdenum the synthesis of proteins is blocked and plant growth ceases. Without it seeds may not form completely and nitrogen deficiency may occur. It has been implicated in gaseous nitrogen fixation and nitrate assimilation.

The pale green rolled leaves are the sign of Mo deficiency. Interveinal mottling of the lower leaves followed by marginal necrosis. The appearance of whiptail is a common molybdenum deficiency disease. Hewitt (1975) reported that appearance of whiptail disease causes chloroplast disorganization. Deficiency of Mo also leads to a drop in ascorbic acid concentration in plants. In groundnut Mo deficiency symptoms first occurs in older leaves. Mo deficient leaves become bright yellow, leaf margin curled and leaves completly collapse. Often lack of Mo reduced chlorophyll concentration in the leaves (Singh, 1999). According to Singh (1994) normal range of Mo in leaf is 0.02 ppm.

Peanut plants require molybdenum in extremely small quantities (Reid and Cox, 1973). It was found that peanut foliage was greener due to more nitrogen when molybdenum or lime was applied (Harries, 1959). Phenolic compounds such as, phenol, resorcinol and parogallol in higher concentration reduced the uptake of nutrients in groundnut plant (Sulaiman and Vijaykumar, 2005). Recently Tuna *et al.* (2007) found that salicylic acid ameliorates the macro and micro-elements of leaves and roots in Maize plants.





However in view of earlier findings about the requirement of Mo in groundnut (Reid and Cox, 1973) the changes in Mo uptake due to SA treatment are critical in Cv. SB-11 than W-44 except 50 ppm SA does. Except 5 ppm treatment SA shows positive impact on accumulation of molybdenum in Cv. W-44 which might be actively involved in nitrogen metabolism as well as to controls molybdenum deficiency diseases.

7. ORGANIC CONSTITUTENTS

a) STARCH

The effect of different concentrations of salicylic acid on starch contents in studied groundnut cultivars is depicted in fig. (32-33). In Cv. W-44 starch contents reduced successively with increase in SA concentration. But higher concentrations i.e. 200 ppm of SA promotes starch content. In cultivar SB-11 this effect of SA on starch content is not uniform. All concentrations of SA increased starch and maximum starch accumulation recorded by 100 ppm SA treatment.

Every green plant synthesizes carbohydrates by the process of photosynthesis to fulfil its energy requirements. Carbohydrates represents one of the most important group of organic compounds in plants as they form a connection between photosynthesis and respiration and thus the yield of plant depends upon its carbohydrate status. Major end products of photosynthetic carbon metabolism are sucrose and starch while glucose is a substrate of respiration. Erythrose, the four carbon sugar is a precurssor of aromatic pathway. Ribose, the pentose sugar is an important part of nucleic acid. Cellulose, the complex polysaccharide plays a structural role. Sucrose is the prim end product of photosynthetic carbon assimilation and in assimilation partitioning. It is the predominate form of reduced carbon transported to the heterotrophic cells. Sugars are osmotically active in nature and thus it also plays an osmoregulatory role. Sucrose is a transient carbohydrate and it is not readily accumulated in cellular storage compartments, it clears that the sucrose might be synthesized and inverted several times before fulfilling its physiological density.

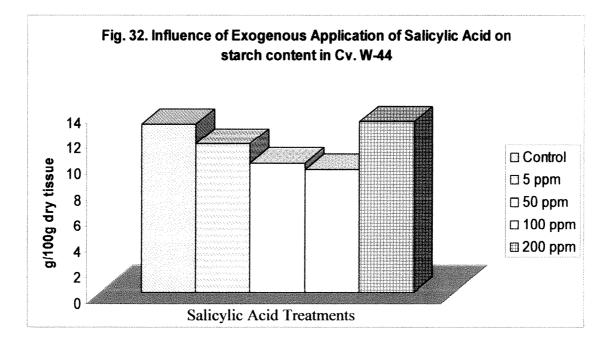
Eventhough groundnut is an oil yielding crop the role of various fractions of carbohydrate in its metabolism is also very important. The changes in carbohydrate content during leaf development in groundnut was analysed by Rane (1987). The souble sugars and starch content increased in leaves with age of plant upto 100 days and 70 days of maturation respectively.

However effect of SA on carbohydrate content is very scanty. Sharma and Lakhvir (1988) observed decreased level of soluble sugars with the foliar application of salicylic acid in Brassica plants. Anandhi and Ramanujam (1997) earlier found that different concentrations of salicylic acid increased the reducing sugars and decreased the non-reducing sugars whereas starch content showed variation in their content in black gram. Sivakumar et al. (2002) documented that foliar application of brassinosteroids, salicylic acid, triacontanol, naphthalene, acetic acid and mepiquat chloride increased the total sugar content in pearl millet Khodary (2004) reported that salicylic acid treatments significantly decreased the contents of soluble sugars than control plants and increased the polysaccharide contents as compared with control and salinity stressed maize plants. He further suggested that salicylic acid might activate the metalic consumption of soluble sugars to form new cell constituents to stimulate the growth whereas it might also be involved to inhibit polysaccharide hydrolyzing enzyme system or accelerate the incorporation of soluble sugars into polysaccharides. El- Tayeb (2005) found that barley grains treated with salicylic acid increased the insoluble and soluble sugars in plants. According to Radwan et al. (2007) salicylic acid treatments significantly increased the carbohydrate contents in ZYMV (Zucchini Yellow Mosaic Virus) infected pumkin leaves.

It is evident from our observation that both the cultivars of groundnut differ in their response to SA application. Leaves stimulate starch content with SA application may be because of stimulation of photosynthetic activity. However except 200 ppm SA other lower concentrations of SA decreases starch content in cultivar W-44 might be correlated with higher respiration rate or its metabolization for pod development.

b) TOTAL NITROGEN AND CRUDE PROTEINS

The effect of various concentration of foliar application of salicylic acid in the leaves of groundnut cultivars (SB -11 and W- 44) is depicted in Table No.7. It can be seen from this table that in both the cultivars of groundnut i.e. W-44 and SB-11, nitrogen and crude protein content increased with application of SA. In cultivar W-44, 5 ppm SA treatment slightly decline total nitrogen and crude protein but further increase in SA concentration steadily increased these compounds content in leaves. The maximum accumulation of total nitrogen and crude protein noticed with 200 ppm SA application in both cultivars.



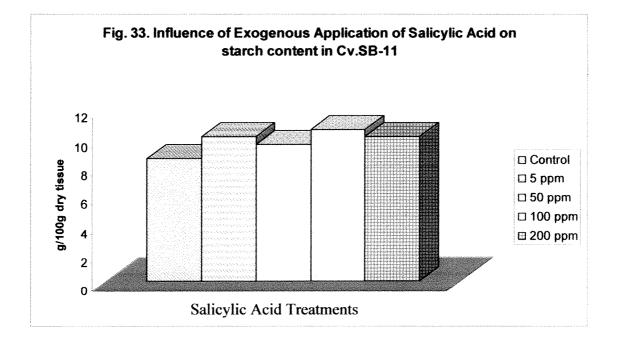


Table 7 : Effect of Foliar application of Salicylic Acid on totalnitrogen content and crudeProteins in groundnut cultivars, W-44 and SB-11

Salicylic acid levels (ppm)	W-44		SB-11	
	Total Nitrogen	Crude Proteins	Total Nitrogen	Crude Proteins
Control	0.24	1.50	0.26	1.62
5	0.21	1.31	0.29	1.81
50	0.28	1.75	0.34	2.12
100	0.33	2.06	0.36	2.25
200	0.35	2.18	0.37	2.31

Values are expressed as g.100g⁻¹ dry tissue Each Value is mean of 3 determinations. Nitrogen (N) is the most important element for plant life. It is an essential component of proteins, hormones, chlorophylls, vitamins and enzymes essential for plant life. It is found in purines and pyrimidines of nucleic acid, RNA and DNA, which are essential for protein synthesis. Nitrogen metabolism is a major factor in stem and leaf growth i.e. vegetative growth. 'N' deficiencies can reduce yield, causes yellowing of the leaves and stunted growth. The nitrogen requirement of groundnut is much higher than cereals, as it contains high proteins and most of the nitrogen requirement is being met through nitrogen fixation in it.

The earlier reports of Srivastava and Jain (1981) noticed that the higher concentration of SA decreases the accumulation of nitrogen in maize seedlings. The total nitrogen levels reduced in *Vigna mungo* plants which has risen from SA presoaked seeds (Jaleel *et al.*, 1998). But majority of reports are available showing increase in nitrogen content in response to SA application.

The application of hormone (NAA) and some nutrients (KNO₃, ZnSO₄, MgSO₄) was found to be increased the 'N' content in groundnut (Naik *et al.*,2005). The increase in nitrogen content by SA treatment in both the groundnut cultivars in our findings are in agreement with the results reported by Sivakumar *et al.* (2002) in Pearl Millet and Alpaslan *et al.* (2005) in maize. According to Singh and Usha (2003) SA maintained nitrogen content in wheat leaves. Similary Singh and Singh (2007) were found that SA causes induction in nitrate assimilation through the induction of total nitrogen. Moussa (2000) reported the interaction between 'N' and 'Mg'. He further noticed that increased 'N' supply to groundnut enhances the uptake of various minerals such as, N, P, K. Ca, Mg, Fe, Mn, Zn and Cu. Our results are also in agreement with this report i.e. the increased nitrogen content due SA application favour uptake of some minerals. Recently Awasthi and Garg (2007) reported increased 'N' content in shoots and roots of maize seedlings by application of different concentrations of SA.

It is clear from the forgoing account that SA shows favourable influences on nitrogen content in both the investigated groundnut cultivars, W-44 and SB-11.