# CHAPTER III

# RESULTS & DISCUSSION

## a) GERMINATION AND SEEDLING GROWTH:

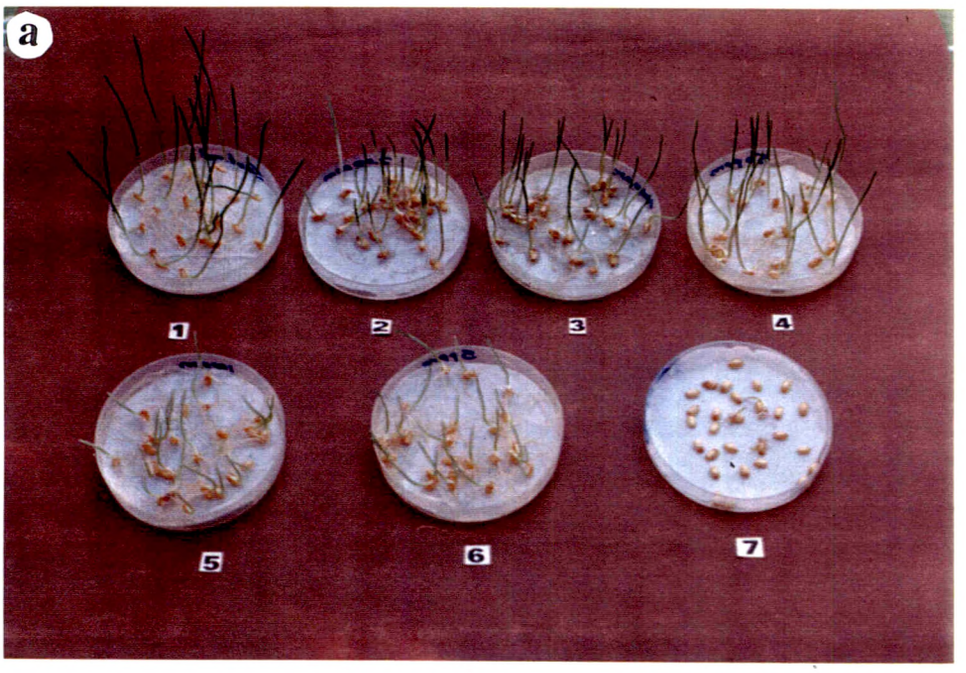
In the Plate No. 2 and 3, Table No. 4 and 5 and Figures No. 3-8 effect of salicylic acid on germination percentage and seedling growth of wheat and moong is recorded. The germination percentage during initial phase is decreased due to higher concentration (above 100 ppm) of salicylic acid. The germination rate is increased slightly in 100 ppm SA treatment in case of wheat seeds during early 24 hrs. In case of moong the germination percentage is increased in 50 ppm SA treatment during first 24 hrs. The germination percentage in moong is considerably decreased with higher concentrations (200 and 500 ppm) of SA. In both the crops after initial lag phase the germination percentage in the SA treated seeds reaches a level similar to that of control at 120 hrs. germination stage. However 500 ppm SA appears inhibitory for germination of wheat seeds even at this stage.

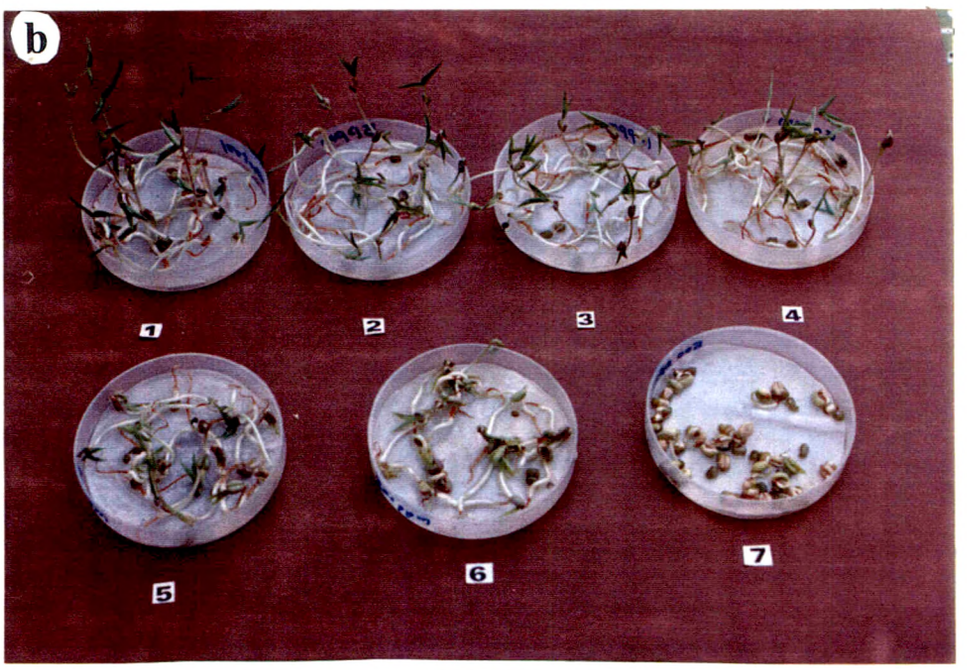
The shoot length and root length of seedlings of both the crop plants are reduced due to salicylic acid treatment (Figures no. 3, 4, 5 and 6). There is no consistent effect of increasing SA concentration on fresh weight of wheat and moong seedlings apart from the fact that at 500 ppm SA treatment, it is significantly reduced (Figures 7 and 8).

In the life cycle of plant, seed germination is an important event. This particular process determines survival of most of plant species on the earth. In view of Mayer and Poljakoff Mayber (1978) germination is a very complex process. The variety of factors including endogenous as well environmental influence seed germination. An increase in germination and enhanced seedling vigour obviously lead to establishment of good crop stand and increase in crop yield. Number of chemicals are used either directly or as pretreatment to improve the seed germination and seedling growth. There are several reports, which indicate increase in germination percentage and seedling vigour by the plant growth regulators and other chemicals. The nitrate at concentration upto 50 ppm

**Plate II**

Effect of salicylic acid on germination of a) wheat & b) moong seeds

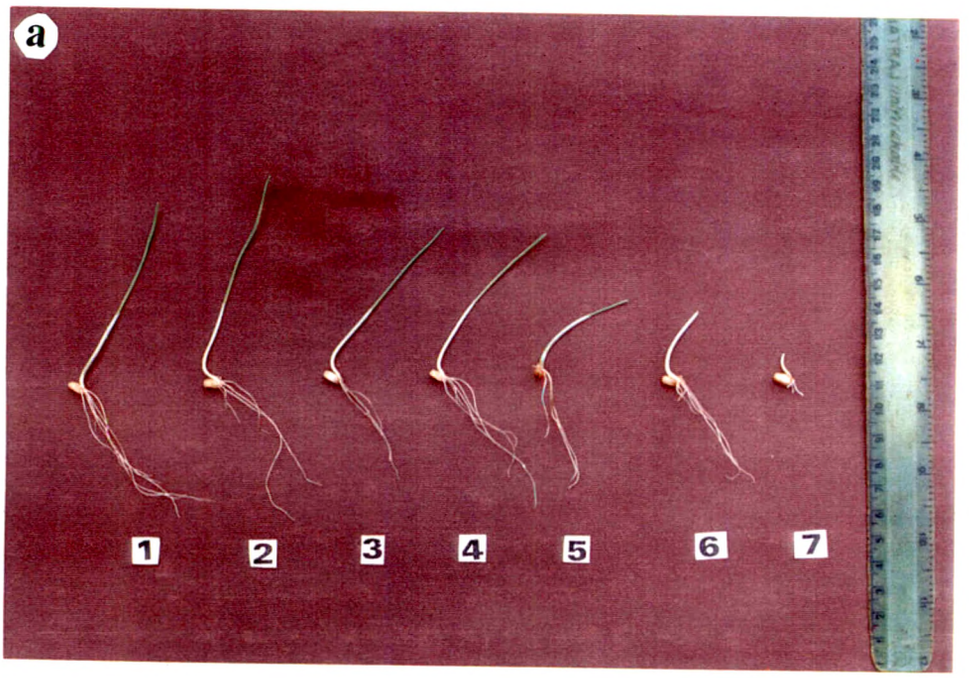



1) Control 2) 5 ppm SA 3) 10 ppm SA 4) 50 ppm SA
5) 100 ppm SA 6) 200 ppm SA and 7) 500 ppm SA

**Plate III**

Effect of salicylic acid on seedling growth of a) wheat & b) moong seeds



1) Control 2) 5 ppm SA 3) 10 ppm SA 4) 50 ppm SA
5) 100 ppm SA 6) 200 ppm SA and 7) 500 ppm SA

Table no. IV: Effect of salicylic acid on germination percentage of wheat seeds.

| Treatments | 24 hrs. | 48 hrs. | 72 hrs. | 96 hrs. | 120 hrs. |
|---|---|---|---|---|---|
| **Control** | 81% | 99% | 99% | 99% | 99% |
| **5ppm SA** | 73% | 94% | 96% | 97% | 97% |
| **10ppm SA** | 85% | 94% | 99% | 99% | 99% |
| **50ppm SA** | 75% | 97% | 100% | 100% | 100% |
| **100ppm SA** | 64% | 95% | 100% | 100% | 100% |
| **200ppm SA** | 51% | 77% | 90% | 95% | 95% |
| **500ppm SA** | 0% | 18% | 26% | 30% | 30% |

Table no. V: Effect of salicylic acid on germination percentage of moong seed.

| Treatments | 24 hrs. | 48 hrs. | 72 hrs. | 96 hrs. | 120 hrs. |
|---|---|---|---|---|---|
| **Control** | 75% | 100% | 100% | 100% | 100% |
| **5ppm SA** | 76% | 100% | 100% | 100% | 100% |
| **10ppm SA** | 71% | 100% | 100% | 100% | 100% |
| **50ppm SA** | 85% | 100% | 100% | 100% | 100% |
| **100ppm SA** | 73% | 100% | 100% | 100% | 100% |
| **200ppm SA** | 65% | 100% | 100% | 100% | 100% |
| **500ppm SA** | 15% | 79% | 88% | 93% | 93% |

Fig.3 Effect of salicylic acid on shoot length of wheat seeding

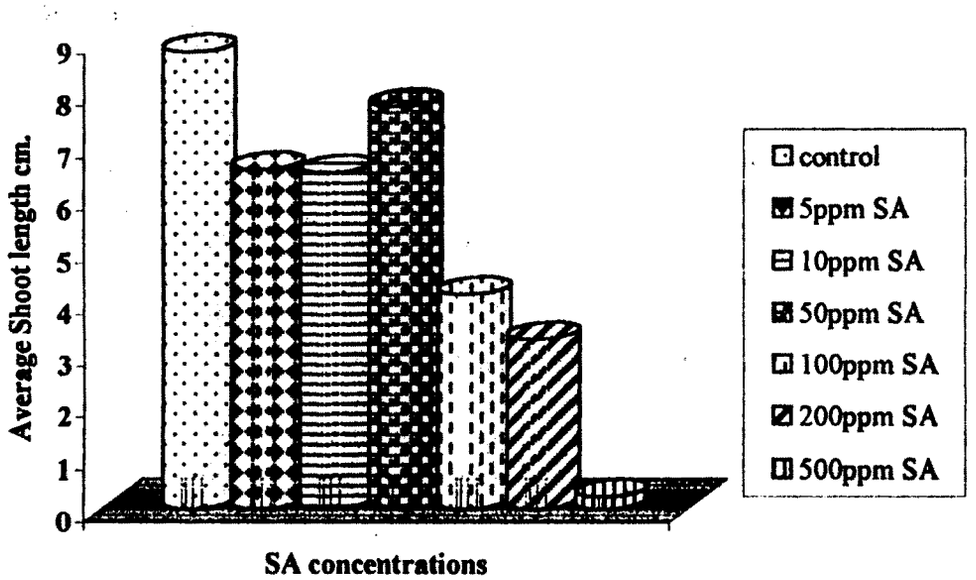



Fig.4 Effect of salicylic acid on shoot lenth of moong seeding

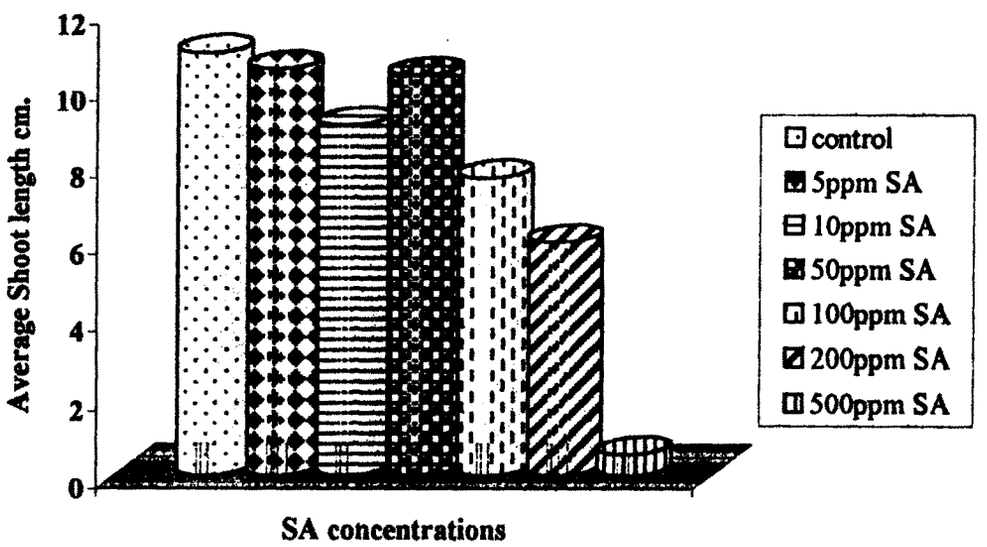


**Fig. 5 Effect of salicylic acid on root lenth of wheat seeding**

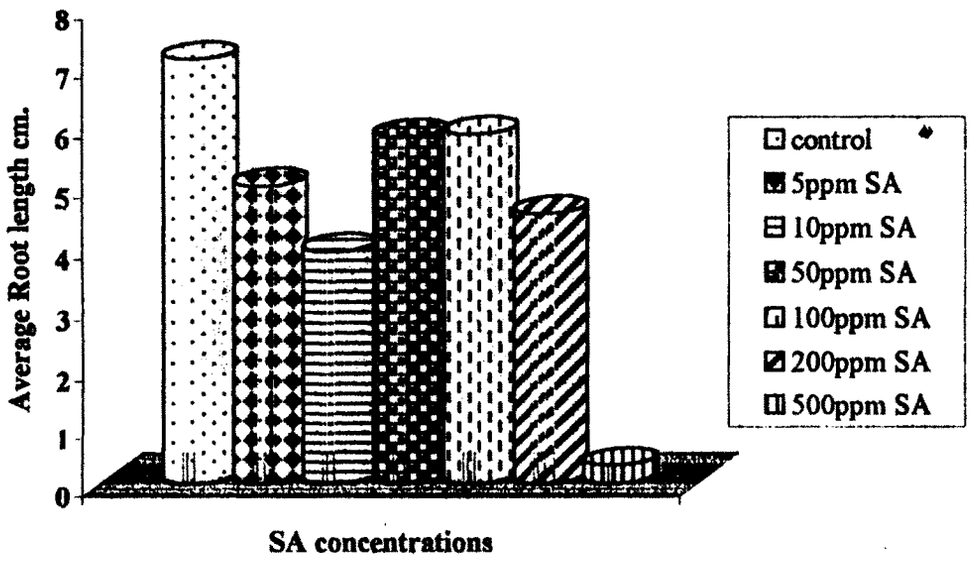



**Fig. 6 Effect of salicylic acid on root lenth of moong seeding**

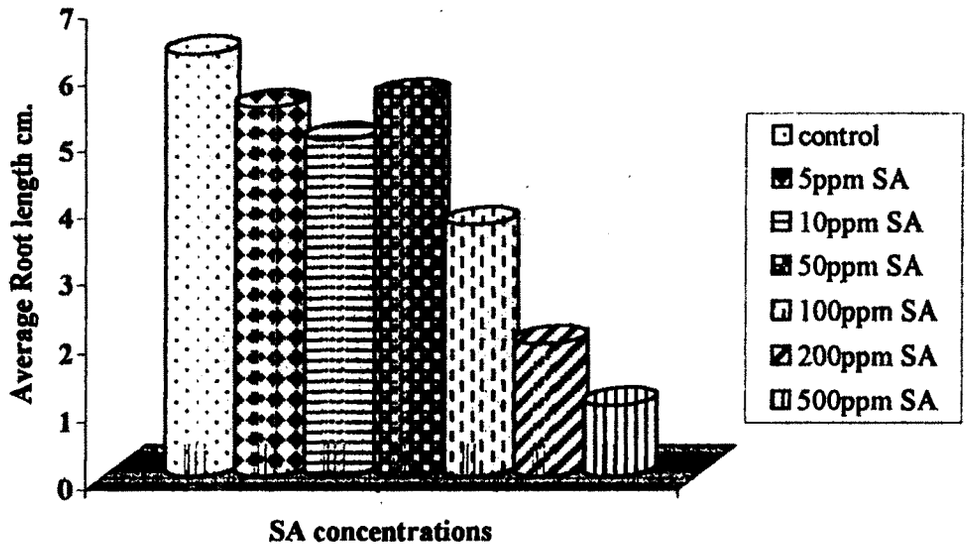


Fig. 7 Effect of salicylic acid on fresh wt. of seedlings of wheat.

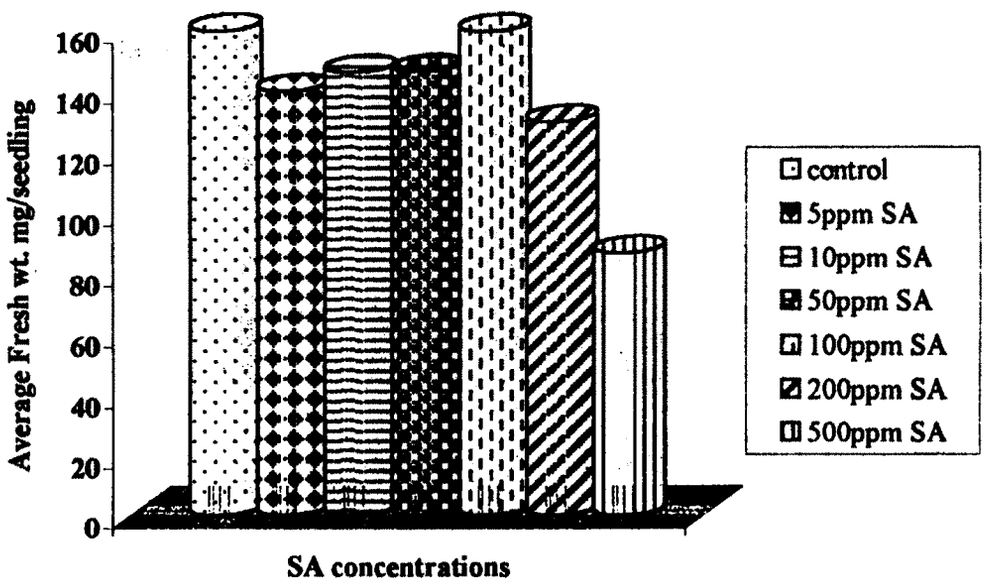
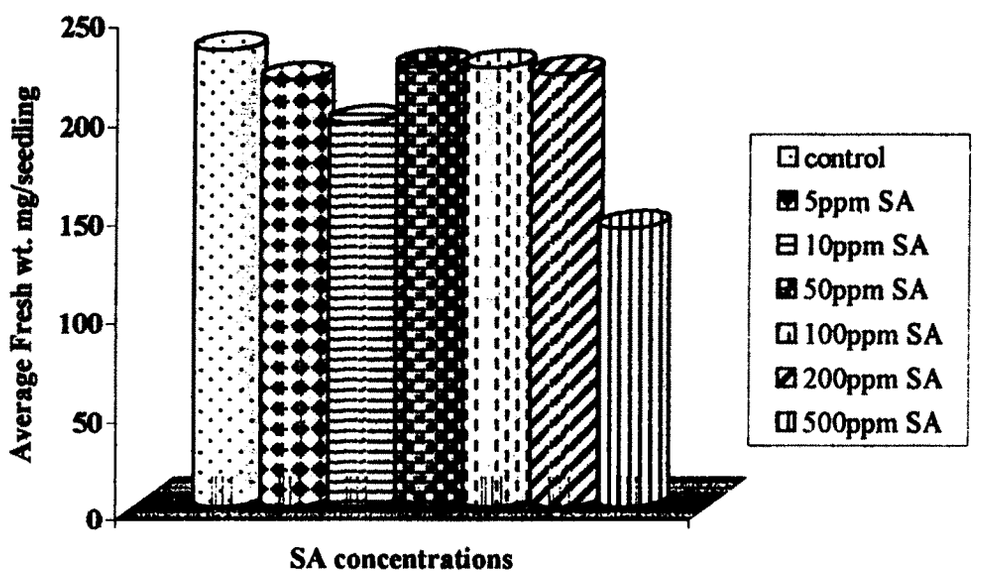



Fig. 8 Effect of salicylic acid on fresh wt. of seedlings of moong.



is reported to promote seed germination in many species (Bewely and Black, 1994).

Vacha and Harvey (1927) for first time showed promotion of seed germination by ethylene. Ketring (1977); Taylorson (1979); Bewley and Black (1982); Ables *et al.,* (1992) also supported this. In 1967 Chinoy noticed increase in germination rate by ascorbic acid. Gibberellic acid, Kinetin and hydrogen peroxide improve germination and seedling growth in one year aged soybean (Saxena and Maheshwari 1979). Gibberellins control and promote the germination (Karseen *et al.*, 1983; Takahashi *et al.,* 1992) while Abscisic acid inhibit the seed germination (Addicott1983). Patel and Saxena (1994) noticed increase in seed germination by gibberellic acid, kinetin, naphthalene acetic acid (NAA) etheral and indole butyric acid (IBA) in green and black gram. They further noticed that $GA_3$, Kinetin and NAA caused increase in seedling growth in both the green and black gram. At the same time, plant growth retardants such as CCC are found to retard seed germination and inhibit the seedling growth (Dencheva and Khsarska, 1988).

Thakur (1977) isolated phenolic growth inhibiting substances from the dormant buds of sugar mapple. He further reported that these phenolic substances inhibited the germination of lettuce. According to Letham (1978) and Harborne (1980) phenolic compounds are involved in growth and metabolism. In *Cajanus cajan,* seed germination was inhibited by phenol and the inhibitory effect of phenol was reversed by gibberellic acid (Tayal and Sharma 1981). Sudheer and Rao (1998) found that caffeic acid gentisic acid, 3, 4-dihydroxybenzaldehyde and ferrulic acid enhanced the growth while p-coumaric acid quercetin and p-hydroxy benzoic acid inhibited growth of *Catharanthus roseus.* Reddy and Rao (1999) noticed increase in height, fresh and dry weight of plant and leaf area of *Nicotiana tabacum* L. *var.* FCV by exogenous application of caffeic acid and ferulic acid. But β-coumaric acid have negative effects. There are very few attempts to study the effect of salicylic acid on seed germination in spite of the

fact that allelopathic potential of this compound is reported in some experiments. Kumar and Tayal (1982) observed that phenolic compounds including SA enhanced growth of plumule and radicle at low concentration but higher concentration had negative effect on these processes in lentil and mungbean. Phenolic compounds and $GA_3$ at 10 and 100 ppm concentration accelerated plumule and radicle growth but the higher concentration (500ppm) of these compounds inhibited the growth in *Cicer arietinum.* They further reported that there is no effect of these chemicals on germination of *Cicer arietinum.* Guan-Linggiang and Scandalios (1994) noticed that SA at 1mM enhanced growth of previously germinated embryos but inhibited seed germination at one day after imbibition.

Asthana and Srivastva (1978) reported increase in maize seed germination by ascorbic acid treatment but salicylic acid in combination with ascorbic acid or salicylic acid alone inhibited the germination of maize seeds. Thus it is evident from these experiments that SA is able to counteract the positive effect of ascorbic acid on seed germination.

Present investigation clearly shows that due to salicylic acid the velocity of seed germination is reduced in both the species at higher concentration. The seedling growth is also affected by salicylic acid. The reduction in shoot growth in both the species is more pronounced than that of root growth. At the highest concentration of SA (500 ppm) the seedling growth of wheat is affected to a greater extent than that of moong.

**b) WATER UPTAKE:**

The effect of SA treatment on water uptake by germinating seeds of wheat and moong is recorded in the Figures 9 and 10. It is clear from the Figures that amount of water taken up is increased in all the treatments when compared with control in germinating seeds of both wheat and moong. The higher water uptake was seen at higher concentration in case of wheat seeds while higher water

**Fig. 9: Effect of salicylic acid on water uptake during wheat seed germination .**

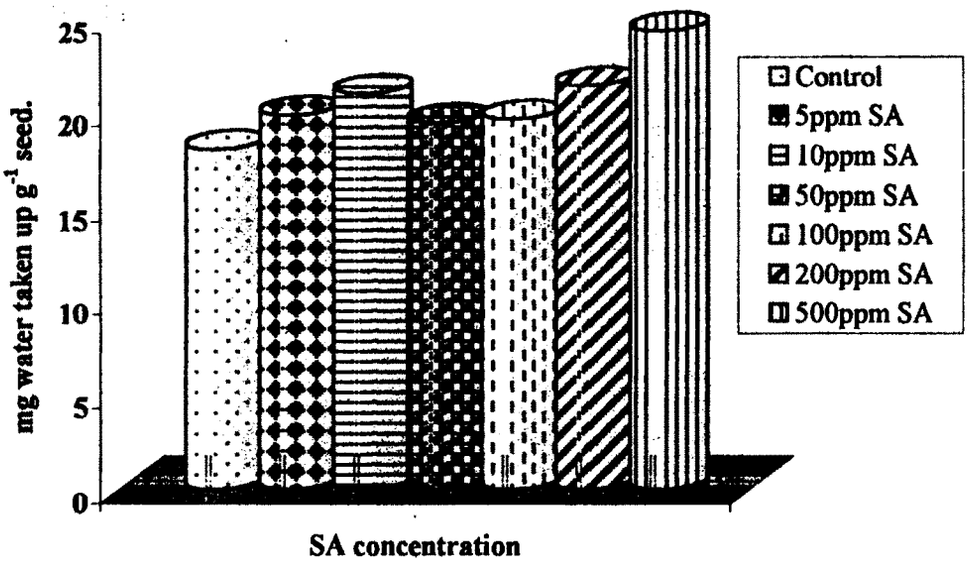
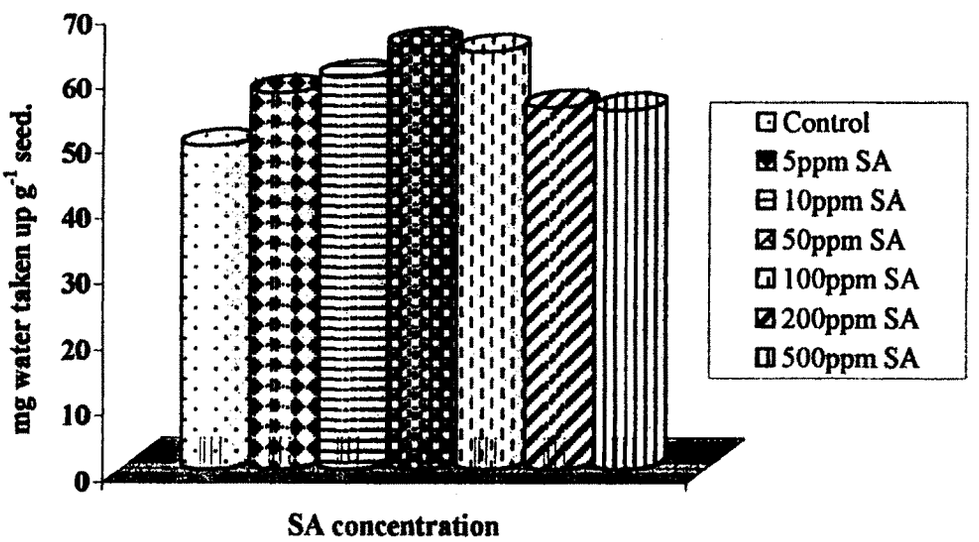



**Fig. 10: Effect of salicylic acid on water uptake during moong seed germination.**



uptake was found at 50 and 100 ppm SA treatment in case of moong. Among different treatments, the highest water content is recorded in seeds treated with 500 ppm SA while in moong it is at 50 ppm SA treatment.

Water is an important constituent of all the biological processes. In the cell about 92% of water is present. It provides aqueous environment for all enzymatic reactions essential to drive the life processes. In photosynthetic tissue water is major electron donor in photosynthetic reaction and this act as a substrate for light reaction. It is also involved in hydrolytic processes. It is essential for growth and a good solvent for translocation of metabolites and nutrients in plants. During the seed germination entry of water is essential for commencement of all the metabolic processes. So the water uptake is the first processes, which occurs during the germination of seed. Imbibition is the first processes in this sense. In view of Vertucci and Leopold (1983) imbibition is nothing but initial wetting of seed tissue. It is followed by establishment of sharp wetting front (Waggoner and Parlange 1976) followed by swelling of the seed and an associated increase in seed water potential as seed water content increases. Seeds have an extremely low water potential which is attributed to their osmotic and matric potential. According to Shaykewich (1973), the osmotic and matric potential may be upto –4000 bar low during initial imbibition of rape, wheat and corn.

The preexisting macromolecules, organelles are reactivated by hydration of seeds. The aqueous medium is necessary for activation, action and synthesis of enzymes, which are involved in metabolic processes. The imbibition of soybean embryonic axis exhibits a reverse sigmoidal water uptake pattern and within 24 hours cotyledons are fully hydrated at 550 g/kg fresh weight and it is not related with seed germination and the embryonic axis maintain higher moisture level (Mcdonald *et al.,* 1988 a ). Constant and changing external water potential affected water uptake and germination in *Cicer arientinum* and *Vicia faba.* (Hadas 1976)

In view of Schopfer *et al.,* (1979) growth inhibitor like abscisic acid stimulate seed dormancy by restricting water uptake of imbibed seed during germination and the active push of embryo expansion which is needed for the growth of embryo. So low water uptake results in failure of germination which may be due to high levels of abscisic acid in the seeds of *Sinapsis alba.* Similar to ABA, SA also inhibits seed germination at very high concentration in both wheat and moong. However the mechanism may be different since process of water uptake is not at all affected by SA treatment.

**c) RESPIRATION:**

Influence of salicylic acid on the process of respiration is depicted in Figures 11 and 12. It is clear from Figures that the rate of respiration in germinating wheat seeds is decreased due to SA treatment. In case of germinating moong seeds the rate of respiration is increased by 5 ppm and 10ppm SA treatments but the higher concentrations of SA have caused a decline in the respiration rate.

It is well known fact that the germination of seed is accompanied by marked increase in respiration. Since respiratory process provides biological energy currency ATP and carbon skeletons for biosynthesis of various metabolites, it is regarded as fundamental metabolic process during seed germination.

Bewley and Black (1978) proposed two distinct patterns of mitochondrial development in imbibed cotyledons of legume. During the process of imbibition the inhibition of mitochondrial protein does not prevent development of mitochondria. The process of repair and activation of immature mitochondria, which are present in dry seed, is going on in germinating seed (Morohashi and Bewley, 1980). Breidebach *et al.,* (1967) found that protein and DNA of mitochondrial fraction increased in pea cotyledons after imbibition. Morohashi *et al.,* (1986) suggested that in legumes two patterns of mitochondrial development

**Fig. 11: Effect of salicylic acid on respiration rate during wheat seed germination.**

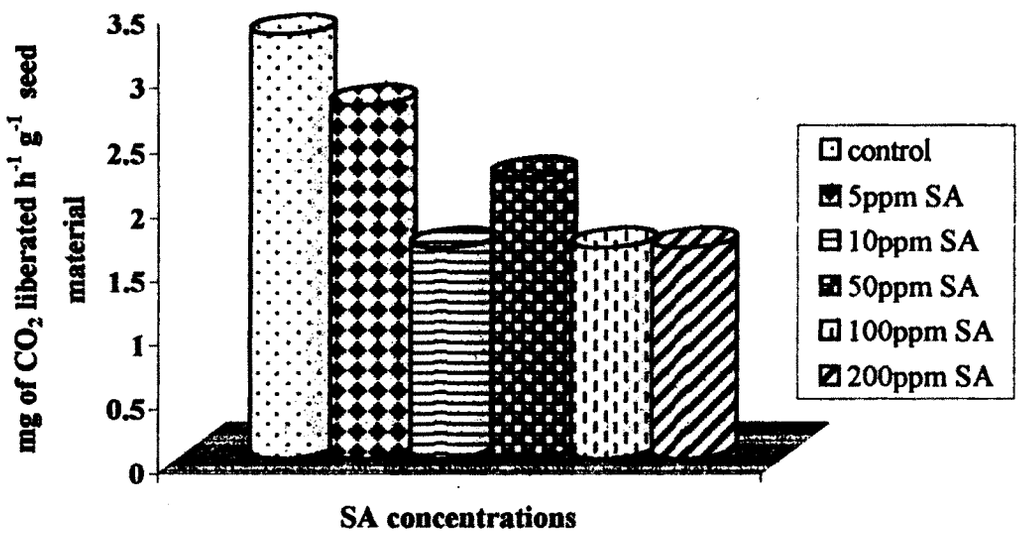
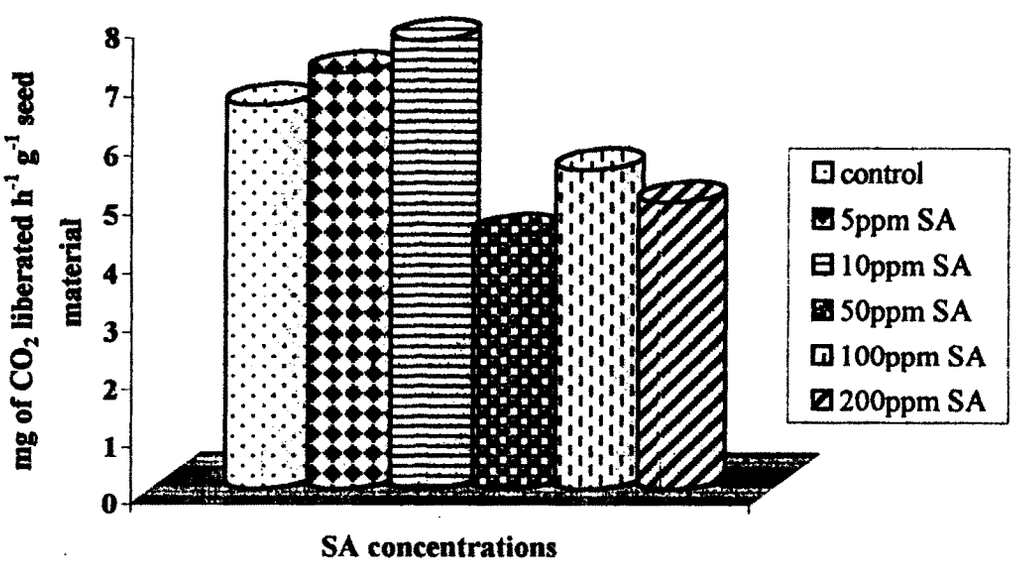



**Fig. 12: Effect of salicylic acid on respiration rate during moong seed germination.**



exist, in imbibed peanut cotyledon respiratory activities increases due to biogenesis of mitochondria, whereas in pea cotyledons the increase in respiratory activities is due to improvement of preexisting organelles. Morohashi (1986) further reported that in starch storing seeds like kidney bean, mung bean, black gram and soybean the mitochondrial activities increased during the first one to two days after imbibition and leveled off thereafter. He further noted that mitochondrial development in starch storing seed is primarily due to improvement of preexisting mitochondria. On the other hand, in lipid storing seeds (cucumber, okra, castor bean) the rate of mitochondrial respiration and activities of mitochondrial enzymes continued to increase. Protein inhibitors are able to stop this, which indicate that *de novo* syntheses of mitochondrial proteins are responsible for the development of organelle activities.

The respiratory process is composed of five distinct phases, glycolysis, pentose phosphate pathway, TCA cycle, electron transport and oxidative phosphorylation and each of the above phases are equally important. Pentose phosphate pathway acts either complimentary or as alternative to glycolysis and activation of this pathway has been implicated in breaking seed dormancy (Roberts, 1972). Organization of mitochondrial assembly during initial phase of germination is very essential for operation of TCA cycle, electron transport and oxidative phosphorylation.

Relatively few studies have been made on influence of growth regulators on respiration during germination. Kato (1956) and Hayashi and Murakami (1958) found that GA caused increase the respiration rate in pea stem. But Waller *et al.,* (1957) found opposite effect in bean plants. Halevy (1963) reported increase in the respiration rate in cucumber hypocotyl and cotyledons. Halevy (1964) found that ethylene enhanced the respiration of banana. In potato tubers also ethylene induced increase in respiration (Solomos and Laties 1975). In cocklebur seeds ethylene stimulated respiration (Esahi *et al.,* 1979, 1990).

Plants contain two pathways for mitochondrial electron flow, the

cytochrome respiratory pathway and the alternative respiratory pathway (Bendall 1958, Bendall and Booner 1971). The alternative oxidase pathway is operating in the heat generating inflorescence. Raskin *et al.,* 1987 identified that salicylic acid is a responsible for the thermogenesis. Exogenous SA has been shown to produce an increase in alternative oxidase specific mRNA in thermogenic spadix tissue from *Sormatium guttatum* (Rhoads and McIntosh 1992). So SA is playing important role in alternative oxidase pathway.

It is evident from the present investigation that SA caused marked decrease in the respiration rate in germinating wheat seeds. Such decrease was also noticed in moong seeds at higher level of SA. An arrest of respiratory process can limit the availability of ATP and thus restrict the growth process in the seedlings.

**d) ENZYME DEHYDROGENASE:**

The influence of SA treatment on enzyme dehydrogenase activity is recorded in Figures 13 and 14. It is clear from the Figures that the enzyme activity is markedly reduced by SA treatment. In case of wheat dehydrogenase activity is reduced by both low (5 ppm) and higher concentrations (100 and 200 ppm) of SA. However the activity is slightly stimulated by 10 and 50 ppm SA treatment.

The enzyme dehydrogenases are oxidizing enzymes, which catalyze electron transfer from the donor to acceptor (other than molecular oxygen). Like oxidases, dehydrogenases are also important in the cellular metabolism. Farkas *et al.,* (1960) and Malca and Zscheile (1964) reported that the activity of dehydrogenases of pentose phosphate pathway and Kreb cycle increases with increase in metabolism of injured plant. Chakravarti and Burma (1959) found that in seeds dehydrogenases are key enzymes of glycolysis, pentosephosphate pathway and Kreb cycle. In glycolysis the glyceraldehyde 3-phosphate dehydrogenase is an important enzyme. Under anaerobic condition alcohol

**Fig. 13: Effect of salicylic acid on activity of enzyme dehydrogenase during wheat seed germination.**

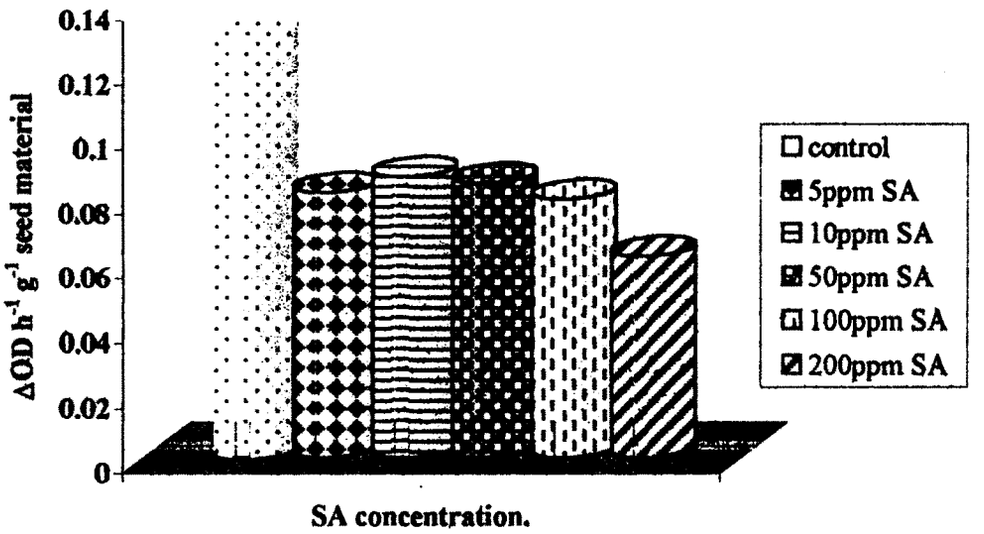
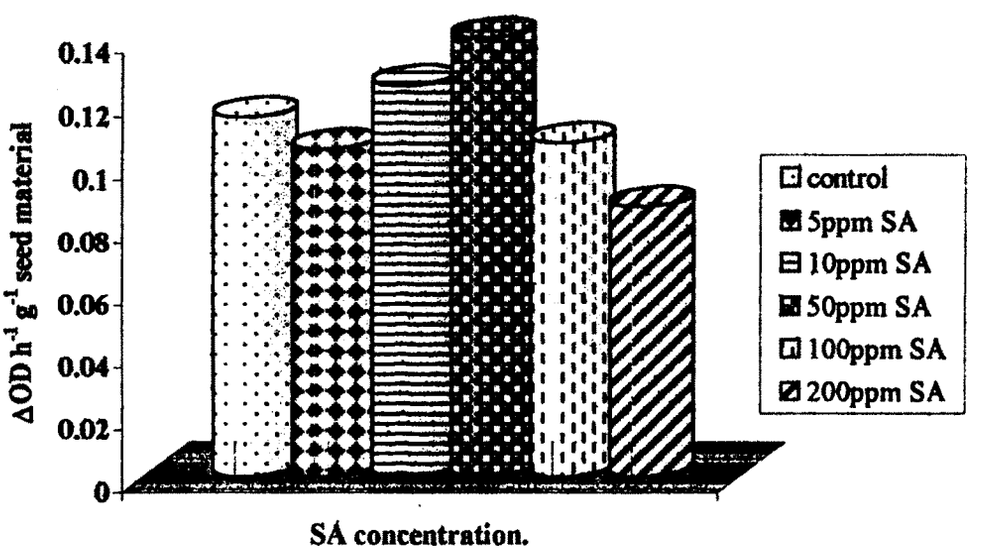



**Fig.14: Effect of salicylic acid on activity of enzyme dehydrogenase during moong seed germination.**



dehydrogenase and lactate dehydrogenase play important role. Pyruvate dehydrogenase complex (PDC) is a large multienzyme complex that catalyzes the oxidative decarboxylation of pyruvate and esterification of the resulting two carbon compounds to CoA and reduction of NAD. Isocitrate dehydrogenase, □-Ketoglutarate dehydrogenase complex, succinic and malic acid dehydrogenase are the important dehydrogenases, which catalyze the Kreb cycle reactions. The enzyme such as NADH dehydrogenase complex plays important role in mitochondrial electron transport. Besides respiration, dehydrogenases are also involved in amino acid metabolism. In view of Roberts (1972) Pentose Phosphate Pathway is involved in dormancy breaking phenomenon during the early stage of germination since as a result of activity of glucose 6-phosphate dehydrogenase and 6-phospho gluconate dehydrogenase, adequate amount of NADPH becomes available for various processes such as fatty acid synthesis. As dehydrogenases are playing important role in respiration there is close relationship between dehydrogenase and respiration rate (Price and Thiman 1954). Perl (1978) demonstrated a system, which seems to direct the ATP synthesis in seeds. The main dehydrogenase activity in seeds as reflected by reduction of tetrazolium is malate dehydrogenase (NADP dependent, EC 1.1.1.37) Moreover during early stage of germination malate dehydrogenase increased which is followed by decrease in its activity after several hours of seed imbibition. It clearly indicates the possible role of malate dehydrogenase in early stage of seed germination. According to Perl and Gloherson (1981) activity of enzyme malate dehydrogenase increases during the last stage of lettuce seed ripening. According to Morohashi (1986) in lipid storing seeds like cucumber, castor bean the rate of mitochondrial respiration and activities of mitochondrial enzymes (Succinate dehydrogenase, NAD-malate dehydrogenase and cytochrome oxidase) increased during the experiment period. Cycloheximide and Chloramphenicol could stop this increase in mitochondrial enzyme activity, which is obiviously due to *de novo* synthesis of these proteins. At the same time

Ovcharov and Akhmedov (1972) investigated the activity of malate, pyruvate, lactate and alcohol dehydrogenase in germinating maize seeds. All these dehydrogenases are present within the dormant seeds. The activities of dehydrogenases increased during early phase of germination. These enzymes are present particularly in the embryo.

The hydrogen atoms from dehydrogenase reduce 2-3-5 Triphenyl tetrazolium Chloride to formazan and it is taken as a measure of enzyme dehydrogenase, which is involved in process of respiration. Formazan is a water insoluble red coloured compound. When TTC comes in contact with the embryo after imbibition production of red colour indicate viability of the seed. Mukherji *et al.,* (1971) suggested this mechanism for testing viability of seed. Sharma *et al.,* (1988) reported that phenolic compounds including salicylic acid inhibited the TTC reduction in isolated *Vigna radiata* embryo axis. But simultaneous application of gibberellic acid could reduce this effect. In the present investigation also production of formazan is considered as measure of dehydrogenase activity and it is clearly evident that the enzyme activity is reduced by SA treatment in germinating wheat seeds. In case of moong seeds the picture is not very clear but higher concentration of SA are inhibitory for dehydrogenase system. These findings are in concurrence with the observations made about respiration.

**e) ENZYME α-AMYLASE:**

The Figures 15 and 16 shows effect of salicylic acid on activity of enzyme α-amylase in germinating wheat and moong seeds. It is clear from the Figures that the activity of enzyme α-amylase is reduced by salicylic acid treatment in germinating wheat seeds. In case of germinating moong seeds the enzyme activity is slightly stimulated in 5 and 10 ppm salicylic acid. The enzyme activity is inhibited by highest dose (200 ppm) salicylic acid.

The macromolecule starch is a major reserve carbohydrate deposited in

**Fig. 15: Effect of salicylic acid on activity of enzyme α-amylase during wheat seed germination.**

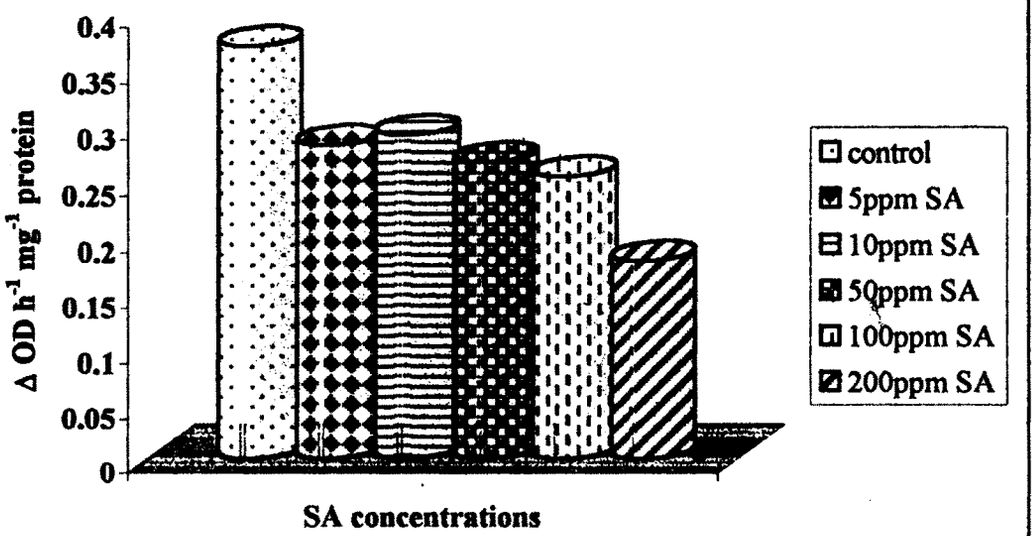
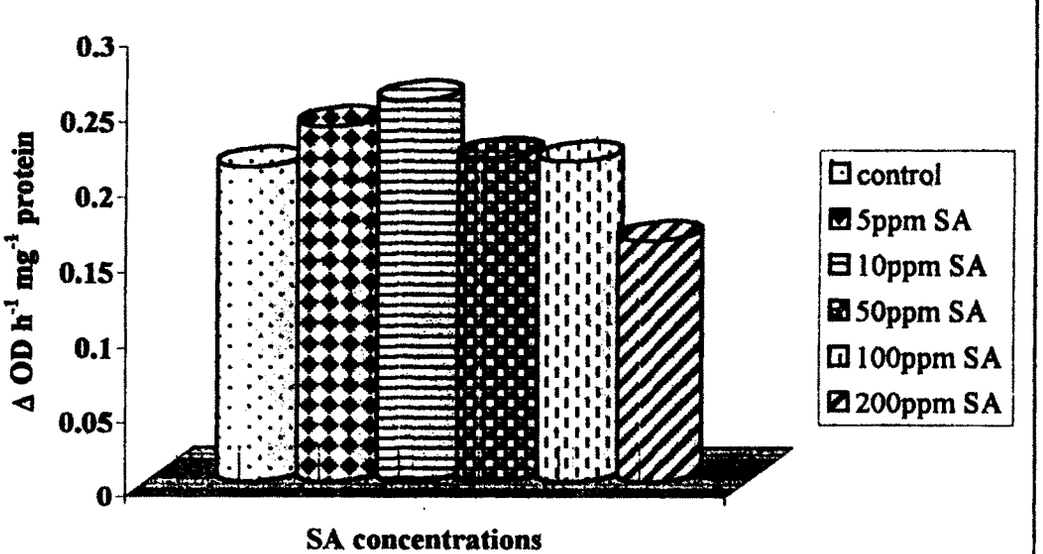



**Fig. 16: Effect of salicylic acid on activity of enzyme α-amylase during moong seed germination.**



most of the seeds. In the process of breakdown of this polysaccharide enzyme α-amylase plays an important role. The α,1-4 bonds of amylose and amylopectin are broken down in random fashion by enzyme α-amylase. Further breakdown proceeds by β-amylase, α-glucosidase and debranching enzyme. Enzyme α-amylase is extensively studied enzyme in cereal seeds. In 1811 Kirchhoff identified a factor which is responsible for modifying starch in wheat extracts (quoted by Fisher and Stein 1960). Payen and Persoz (1833) isolated partially purified α-amylase. The origin of α-amylase and other starch mobilizing enzymes within the cereal grain was a matter of controversy among the botanist at the end of last century. According to Haberlandt (1980) aleurone layer was the source of diastase (α-amylase) in germinating grains. According to Halmer (1985) scutellum is an important site of α-amylase production in rice and sorghum seeds. But in barley seed scutellum α-amylase synthesis and secretion occurs from aleurone layers (Briggs 1973). Akazawa and Hara (1978) noticed that barley seed scutellum plays primary role in α-amylase production. So according to Bewley and Black (1985) enzyme α-amylase is synthesized *de novo* into two specific tissues scutellar epithelium and aleurone layer in cereal seeds.

According to Kirsop and Pollock (1957) rise in barley α-amylase activity of the endosperm depends on presence of embryo. Removal of embryo results in decrease in enzyme activity. Yomo and Varner (1973) and Pleg (1985) demonstrated effect of gibberellic acid on *de novo* synthesis of α-amylase. In 1984 Jones and Carbonell noticed development of four isozymes of α-amylase during seed germination. In the experiments of Wilson (1971) requirement of gibberellic acid was evident for the production of enzyme α-amylase and its light regulation.

Abscisic acid (ABA) inhibited the $GA_3$ stimulated activity of enzyme α-amylase (Chrispeels and Varner 1967). But according to Hodth and Varner (1976) ABA inhibits the $GA_3$ induced α-amylase synthesis by operating at

transcriptional level.

Gibberellic acid and salicylic acid induced amylase activity in leaves of *Impatiens balsamina* (Kumar and Nanda 1981b). But in view of Kumar *et al.*, (1982) phenolic acids are potential inhibitors of plant amylases. Salicylic acid, caffeic acid and gallic acid inhibited plant amylases *in vitro*. Kumar *et al.*, (1982) further reported that inhibition of amylase by phenolic acid was non-competitive type. Low concentrations of $GA_3$ and phenolic acids promote amylase activity (Sharma and Tayal 1984). In case of moong seed we have also noticed similar trend. But the enzyme system in wheat appears more sensitive to salicylic acid (Figure 11). Since wheat is a starchy cereal inhibition of α-amylase activity may pose limitations on availability of respiratory substrate glucose for the germination process.

**f) ACID AND ALKALINE PHOSPHATASE:**

The effect of SA on activities of enzyme acid phosphatase (Apase) and alkaline phosphatase in germinating wheat and moong seeds is shown in Figures17, 18, 19 and 20. It is clear from the Figures that the acid phosphatase activity is decreased, in germinating seeds of wheat due to all doses of SA and on the other hand in case of germinating moong seeds SA treatment has caused an increase in the enzyme activity. Activity of enzyme alkaline phosphatase is increased only at 5 and 100 ppm SA treatment while further higher concentrations of SA have caused reduction in the enzyme activity in germinating wheat seeds. But in case of germinating moong seeds there is no consistency in the effect of SA on the behavior of enzyme alkaline phosphatase.

Phosphorus is an indispensable nutrient for all the living organisms. It is one of the important constituent of nucleic acids, phospholipids and number of other biochemicaly important molecules such as ATP. It is required by cell in large amounts. Deficiency of Phosphorus frequently limits the growth of the plant. Phosphorus is assimilated in the form of orthophosphate anion (Pi). For the

**Fig.17: Effect of salicylic acid on activity of enzyme Apase during wheat seed germination.**

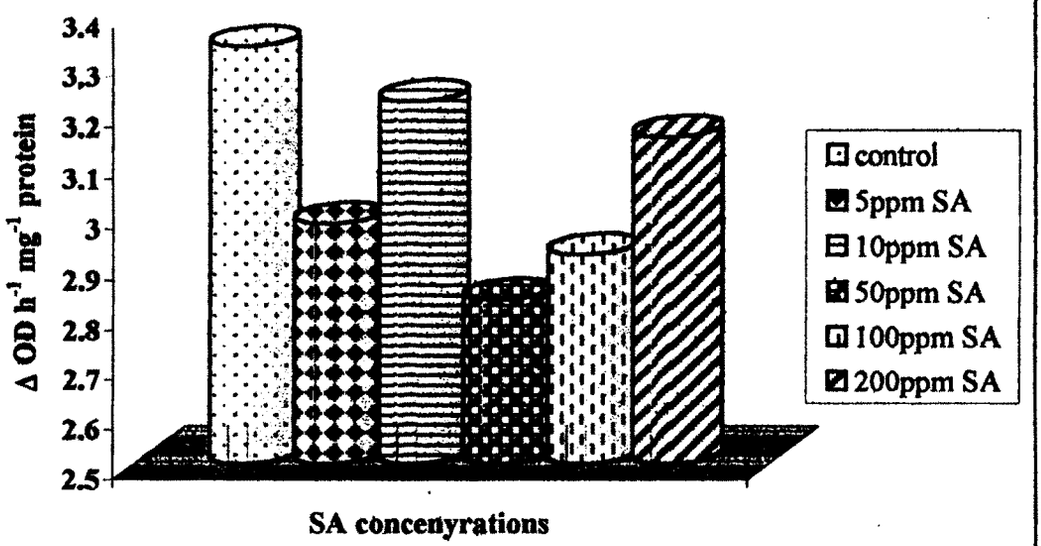



**Fig. 18: Effect of salicylic acid on activity of enzyme Apase during moong seed germination.**

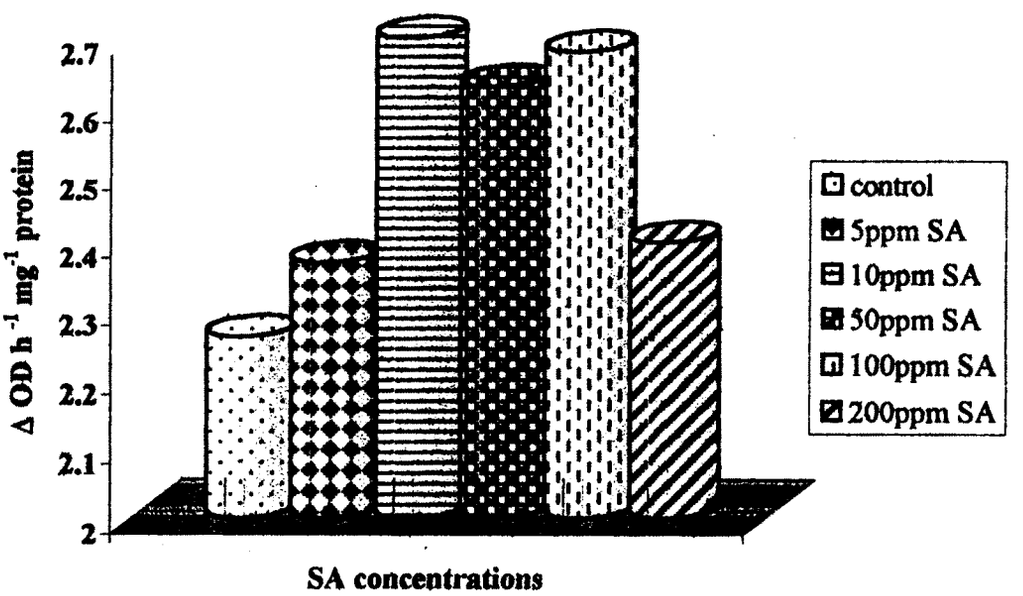


**Fig. 19: Effect of salicylic acid on activity of enzyme alkaline phosphatase during wheat seed germination.**

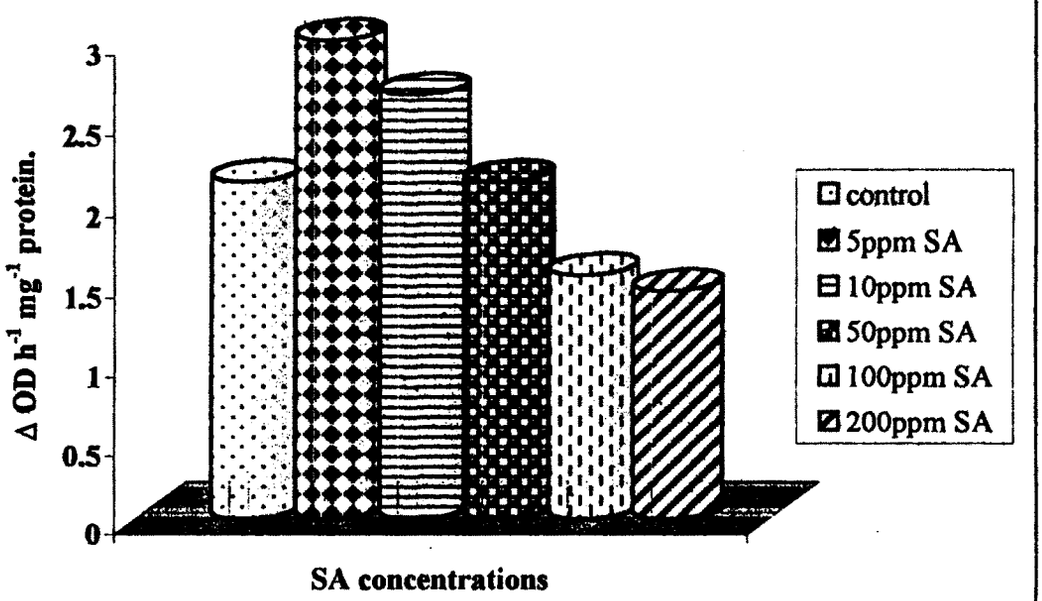
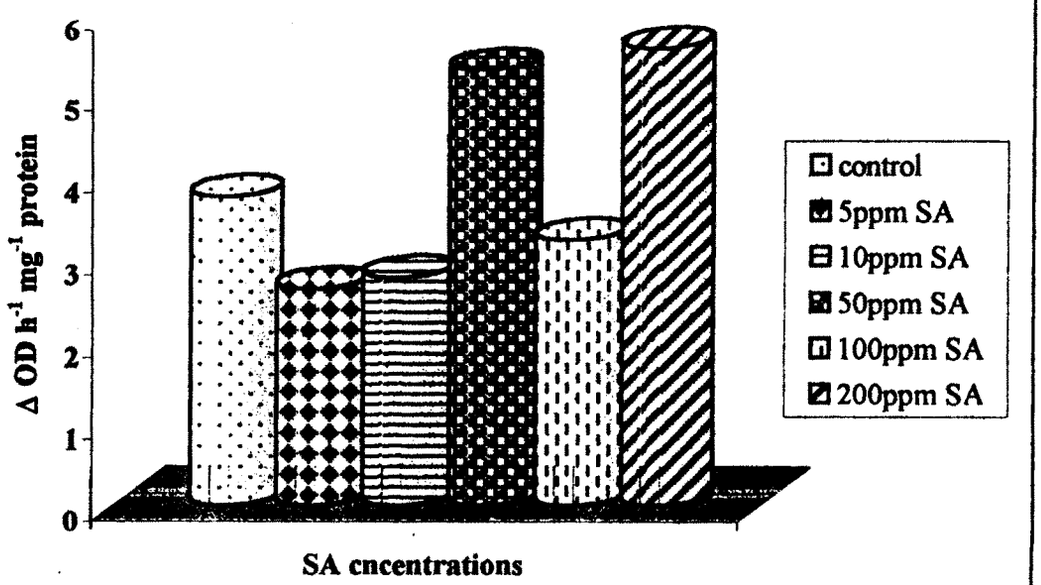



**Fig. 20: Effect of salicylic acid on activity of enzyme alkaline phosphatase during moong seed germination.**



utilization of phosphorus special class of enzyme system is active in the living organisms. These are phosphatases which hydrolyse the orthophosphate anions from orthophosphate monoesters in thermodynamically favourable system (Vincent *et al.*, 1992). Depending upon catalytic activity in the pH optimum, below or above seven phosphatases have been traditionally classified as acid and alkaline phosphatases (Vincent *et al.*, 1992) Alkaline phosphatases in contrast to acid phosphatases (Apase) shows substrate specificity. Examples of this are fructose, 1,6-biphosphatases and sucrose 6 phosphatase. These enzymes are involved in sucroneogenesis. Though Apases do not show substrate specificity then also these are classified on basis of their substrate specificity. First type is a 'Non-specific' enzyme (no substrate specificity) which are found in variety of species and tissues and which shows diversity in physical properties, are playing important role in transport and recycling of Pi. The second types of Apases are specialized enzymes e.g. 3-phospho glycerate phosphatase of maize leaves. (Randall and Tolbert 1971).

In view of Richter *et al.*, (1972) acid phosphatase may play some role in breaking of seed dormancy. Several worker have noticed increase in enzyme acid phosphatase during germination. During germination of the pea the membrane bound acid phosphatase activity was increased (Masatoshi and Asahi 1973). The development of acid phosphatase is singlephasic in the germinating pea seeds (Barker *et al.*, 1974) In germinating seeds of *Vigna mungo* also the activity of the enzyme acid phosphatases increases considerably (Tamura *et. al* 1982).

In coleorhiza and scutellum the enzyme activity was high, while the endosperm had no detectable enzyme activity in wheat seeds (Price and Ey 1970). Colson (1979) found little or no activity in endosperm of *Allium sepa*. He found that after three hours of imbibition acid phosphatase was detectable in the aleurone grains. *De novo* synthesis of two isozymes of acid phosphatase was responsible for increase in activity of this enzyme in germinating pea seeds

(Johnson *et al.,* 1973). Embryoless wheat seeds contain two or more isozymes (Takashi *et al.,* 1981). Janwoski and Iguacio (1977) tried to correlate germination capacity and activity of acid phosphatase and he suggested that the activity of this enzyme is good indicator of germination.

Few reports are available regarding the behavior of alkaline phosphatase during seed germination under saline condition. In pea seedling the activity of alkaline phosphatase was found to be decreased by salinity (Weimberg 1970). In halophytic spinach also enzyme activity decreased due to salinity (Ahmad and Hug, 1974). According to Rajasekhar and Sopory (1985) phosphatases (acid and alkaline) are phytochrome controlled. In 1986 Karadge noticed decrease in alkaline phosphatase activity due to potassium deficiency as well as toxic dose of potassium. Sengupta *et al.,* (1986) found increase in alkaline phosphatase activity in wheat seeds treated with malathion.

Hormonal regulation of Apases activity during seed germination is evident in experiments of several works. The treatment of GA on embryoless wheat resulted in activation of new isozyme of acid phosphatase. According to Bray *et al.,* (1979) increase in acid phosphatase during seed germination was due to *de novo* synthesis of it. Increase in activity of acid phosphatase caused by gibberellic acid was attributed to GA regulated *de novo* synthesis of the enzyme (Bhargava and Sacher 1987). According to Gibson and Ullah (1988) in the ungerminated seeds small amount of acid phosphatase is present. Decrease in phytic acid (seed phosphate reserve) due to increase in acid phosphatase results in germination of seed.

Deleo and Sacher (1970) reported that abscisic acid stimulated the acid phosphatase activity, which accompanies fruit ripening. Chidrawar (1996) reported that salicylic acid ascorbic acid, CCC and ethephon pre treatments accelerated the acid phosphatase activity in germinating soybean seeds.

In moong and wheat seeds we can notice an opposite behavior of enzyme acid phosphatase in response to salicylic acid treatment. In moong there is

considerable increase in enzyme activity due to SA treatment especially at 10, 50, and 100 ppm. concentrations. Thus SA increases availability of Pi for the developing embryo axis through increase in acid phosphatase in moong. However same cannot be said about wheat.

Not much attention has been paid to the fate of alkaline phosphatase during seed germination in different crops. However it is quite possible that this enzyme may play a role in phosphorus metabolism in some cellular compartments where the cellular pH is above 7. The activity of the enzyme is stimulated by lower concentration of SA in wheat while in moong seeds no consistent pattern is noticeable. Thus it is clear that acid and alkaline phosphatases in the two species respond differently to the treatment of salicylic acid.

**g) ENZYME NITRATE REDUCTASE:**

Effect of different concentrations of SA on nitrate reductase activity of germinating wheat and moong seeds is depicted in Figures 21 and 22. It is evident from Figures that the activity of NR is stimulated by SA treatment in wheat, the stimulation being maximum at 50 ppm SA. The higher concentrations (above 100 ppm) of SA have also caused stimulation of NR activity in germinating moong seeds.

Enzyme nitrate reductase catalyzes the first step of nitrogen assimilation and this enzyme is very important in higher plants. The enzyme system consist reduced pyridine nucleotide (NADPH or NADH) as an electron donor, flavin-adenine dinucleotide (FAD) and molybdenum, According to Guereo *et al.,* (1981) during nitrate reduction electrons are directly transferred from molybdenum to nitrite. The activity of this enzyme is a rate limiting step in nitrogen assimilation as this enzyme determines over all assimilation of nitrate. In view of Austin *et al.,* (1978) there is positive correlation between leaf nitrate reductase and growth rate. The activity of nitrate reductase is also correlated with

**Fig. 21: Effect of salicylic acid on activity of enzyme nitrate reductase during wheat seed germination.**

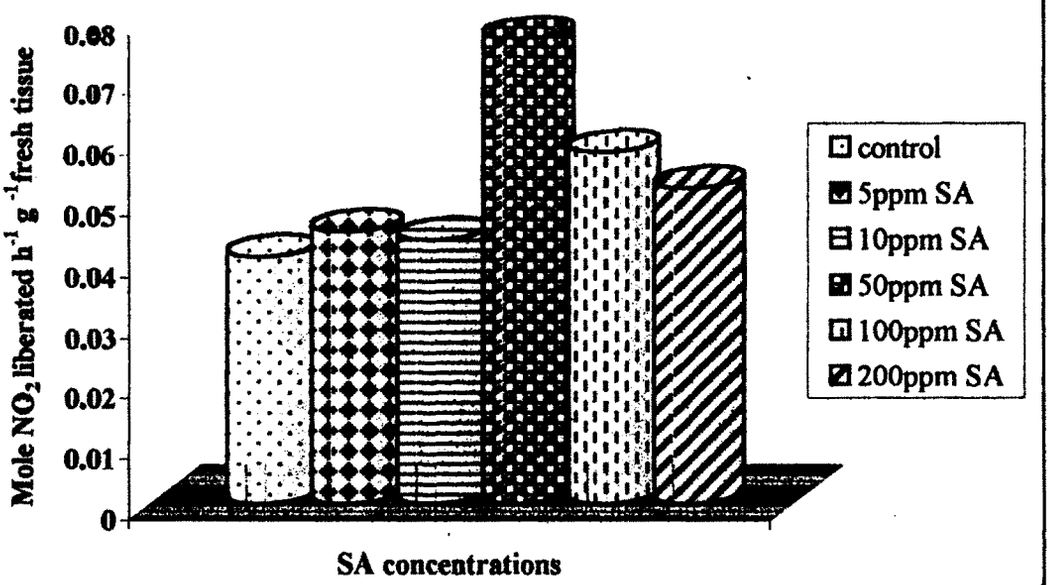
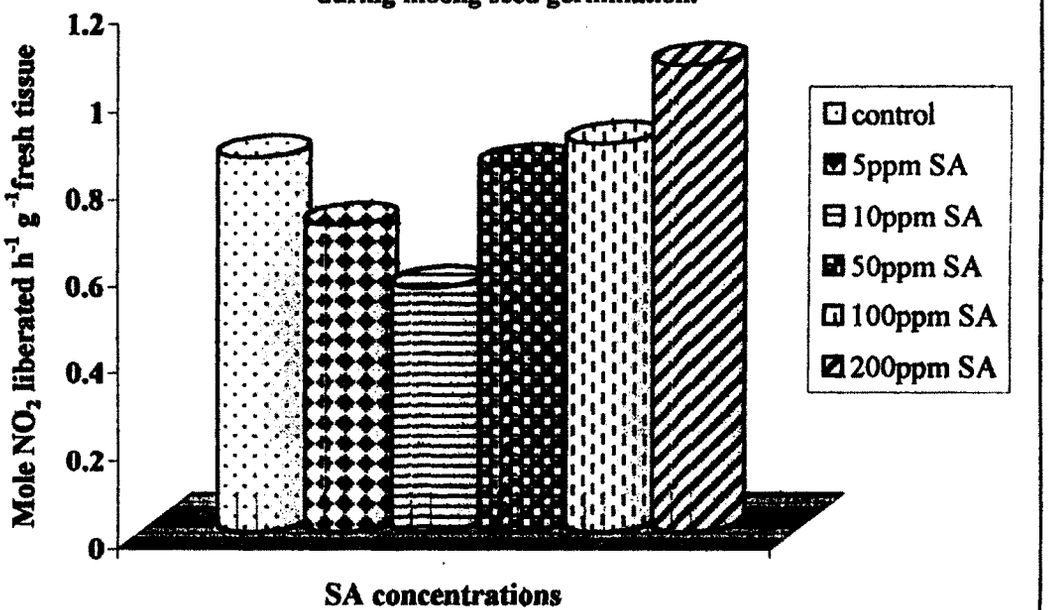



**Fig. 22: Effect of salicylic acid on activity of enzyme nitrate reductase during moong seed germination.**



yield (Kumar *et al.,* 1987).

The activity of nitrate reductase is affected by number of growth regulators. Knypal and Knypal (1974) noticed induction of nitrate reductase activity by benzylaminopurine, succinic acid, 2-dimethylhydrazide, chloramphenicol and chlorogenic acid in seedlings of rice. Bhadre (1983) reported increase in NR activity by CCC pretreatment. Presowing soaking treatment of cottonseeds with CCC causes increase in NR activity of leaves and decrease in roots of cotton seedlings. Ethylene caused increase in membrane permeability might be enhancing nitrate reductase activity by promoting availability of storage $NO_3$. Increased NR activity was also found in CCC and kinetin pretreated groundnut plants under stress condition (Kutwal, 1989). GA in combination with cytokinin caused increase in NR activity in tobacco (Lips and Roth, 1969). IAA also induced NR activity but the effect of growth substances on enzyme is not due to stimulation of general protein synthesis. Abscisic acid had no effect on NR in cotton (Radin and Trealese 1976), but in *Phaseolus aconitifolium* ABA enhanced activity of NR (Sankhala and Huber 1975). Mishra *et al.,* (1995) noticed that the polyamines spermine, spermidine, putrescine and cadavarine caused an increase in NR activity in leaves of *Brassica campestris.* The nitrate reductase activity is increased in first 12 hours and then declined due to simazine treatment in wheat seedlings (Jarzynska and Buczek, 1984). Phenolic compounds also induce enzymes of nitrogen metabolism (Malik *et al.,* 1986). In groundnut NR activity was enhanced by phenolic compounds (Kaur 1987).

Presowing soaking treatment with ascorbic acid and ascorbic acid in combination with salicylic acid enhanced nitrate reductase activity in maize seedlings (Asthana and Srivastava, 1981a). But they further noticed that salicylic acid alone-inhibited NR activity. But Jain and Srivastava (1981b) found increase in NR activity in roots of maize seedling by salicylic acid. Among various phenolic acids salicylic acid induced NR activity but caffeic acid and atonic acid were more effective in this respect (Sharma *et al.,* 1984). Low concentration of

SA enhanced NR activity in *Vigna mungo* (Jaleel *et al.,* (1998). Ramanujam *et al.,* (1998) also noticed increase in NR in blackgram due to SA presowing soaking treatment. But foliar spray of salicylic acid also stimulated NR activity in soybean. (Kumar *et al.,* 1998), Pathmanabhan and Thangaraj 1998).

The enzyme nitrate reductase is substrate inducible enzyme. Its activity depends upon several genetic, ontogenic and environmental factors including nitrate supply and light intensity (Beevers, 1969, Srivastava 1980). The process of transpiration is necessary link in hormonal regulation and substrate induction of NR activity in corn cockle embryos (Kuznetsov *et al.,* 1986). Sinha and Nicholas (1981) noticed that enzyme NR is controlled by availability of nitrate, inhibition of protein synthesis and reduction in availability of NADH. In the present investigation the seeds were allowed to germinate in medium containing nitrate. Hence availability of nitrate was not factor which can influence the NR activity in this system. In both wheat and moong, SA treatment has caused stimulation of the enzyme activity and this may be possibly due to favorable influence of SA on enzyme protein synthesis.

**h) ENZYME CATALASE:**

Effect of salicylic acid treatments on the activity of enzyme catalase in germinating seeds of wheat and moong is recorded in Figures 23 and 24. It is evident from Figures that catalase activity is increased by treatment of 5 ppm SA in germinating wheat seeds. In case of moong enzyme catalase activity increased by 5 and 10 ppm SA treatment. In all further SA treatments the enzyme activity is decreased considerably.

Catalase plays vital role in redox reaction. It is important $H_2O_2$ degrading enzyme. Catalase oxidizes substrates other than $H_2O_2$ (Kermer, 1970). As catalase has more affinity for $H_2O_2$, enzyme regulates level of $H_2O_2$ in plant tissue (Grinberg 1971). Catalase is important enzyme of glycolate pathway which catalyses breakdown of $H_2O_2$ produced during the oxidation of glycolate

**Fig. 23: Effect of salicylic acid on activity of enzyme catalase during wheat seed germination.**

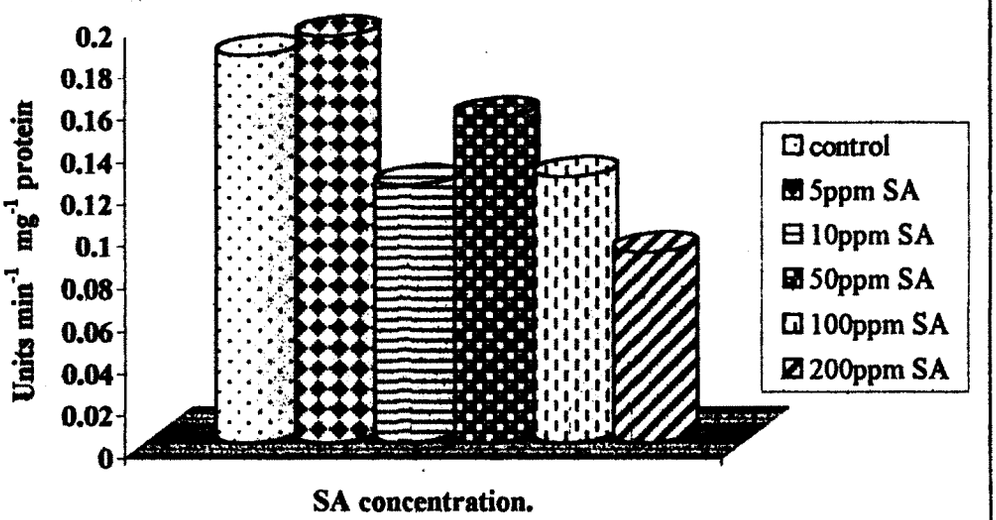
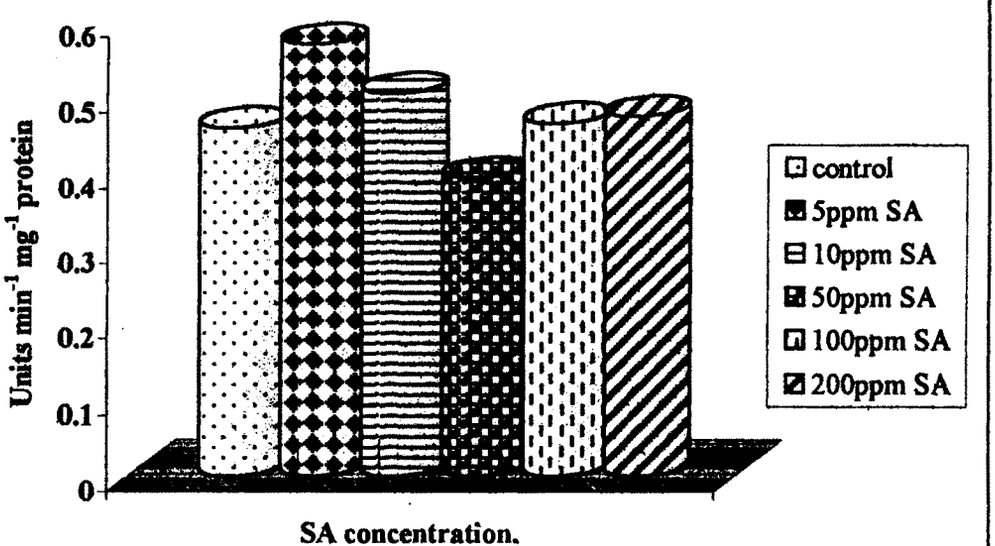



**Fig. 24: Effect of salicylic acid on activity of enzyme catalase during moong seed germination.**



(Tolbert 1971). Besides photorespiration $H_2O_2$ is also produced during β-oxidation. Hydrogen peroxide is a very harmful metabolite in cells, as it is very important casual factor in oxidative stress.

From superoxide radicals, which are produced due to altered electron transport or in light reaction enzyme superoxide dismutase brings about synthesis of hydrogen peroxide. $H_2O_2$ and superoxide radicals are relatively stable but in presence of metal ions the superoxide radicals and hydrogen peroxide can react to form hydroxylradicals. Hydroxylradicals are most reactive species known to chemistry, which cause lipid peroxidation, denaturation of proteins, mutation of DNA. Hydrogen peroxide also oxidizes sulphahydral groups, which results in denaturation of number of enzymes. So catalase is very important enzyme involved in protective mechanism against oxidative stress. It is emphasized by Willekens *et al.,* (1997) that catalase is a sink for $H_2O_2$ and is indispensable for stress defense. They noticed that catalase deficient tobacco plants were markedly more sensitive to light, paraquat, salt and ozone stress but not to chilling stress at low temperature.

During germination also catalase plays important role. According to Nanda (1950) catalase activity is correlated with germination capacity. Nanda (1950) and Chikanse (1953) reported a positive correlation between catalase and seedling growth. Vora *et al.,* (1976) also supported this view. Mahely (1968) noticed that after initial decrease activity continuously increased up to eleven days during almond seed germination.

During rice seed germination catalase activity increased upto 96 hours and then enzyme activity declined (Mukherji and Paul 1971). Palmaino and Juliano (1972) in contrast to above observations found continuous increase in catalase upto six days of rice seed germination. During germination of *Coffea arabica* catalase activity more than doubled (De Oliveira *et al.,* 1976) Hendricks and Taylerson (1974) observed that nitrates, nitrites, hydroxylamine, ammonium Chloride, potassium cyanide and azid induced inhibition of catalase resulted in

breaking of dormancy of *Amaranthus* and *Lactuca*. But many workers do not accept this hypothesis, as in several non-dormant seeds activity of catalase is higher.

Plant growth regulators also affect activity of enzyme catalase. Magdon (1967) found that GA enhanced the catalase activity in *Cicer arientinum* (Mehta *et al.,* 1974). They further noticed that GA could reverse the effect of morphactin. In germinating soybean ascorbic acid, GA and GA in combination with maleic hydrazide caused increase in catalase activity but maleic hydrazide alone showed inhibitory effect. Vora *et al.,* (1976) noticed stimulation of catalase by GA pretreatment in *Sesamum* seedling. Maleic hydrazide and CCC inhibited catalase activity while GA stimulates it (Sangeeta and Varshney 1991). In *Ipomea purpurea* seedling kinetin reduced the enzyme activity.

Phenolic compounds also influence activity of catalase. Catalase activity in intact primary leaves of bean was decreased by phenolic compounds (Chary and Rao 1997). Decrease in catalase activity by quinones in leaf discs of radish was noticed by Chary and Rao (1998). Chen *et al.,* (1993) found that SA binding protein is SA inhibitable catalase in tobacco. Sanchez and Klessig (1994) also supported this. Guan L. and Scandalios (1994) noticed that SA at 1 mM enhanced growth of precociously germinated embryos but on the contrary inhibited seed germination at one day after imbibition. They further noticed that Cat 2-transcript accumulation was inhibited at this stage and concluded that SA is probably not a direct signal for induction of the catalase genes. Recently Mishra and Choudhary (2001) found SA induced decrease in catalase activity in roots and shoots of rice. Similar to rice, in case of wheat also we have noticed a decline in catalase in response to treatments of SA above 5ppm. This can increase the susceptibility of seeds to oxidative stress and cause growth reduction of the seedlings. On the other hand the catalase enzyme system in moong appears to have greater stability than that of wheat since there is no marked reduction in enzyme activity in response to SA treatment.

**i) ENZYME PEROXIDASE:**

Effect of different concentrations of SA on activity of enzyme peroxidase of germinating wheat and moong seeds is shown in Figures 25 and 26. It is clear from Figures that the activity of enzyme peroxidase is inhibited by salicylic acid treatment in germinating seeds of both crops.

Enzyme peroxidase represents another important antioxidative enzyme system, which is extensively studied and understood. The enzyme found to be present in various sub cellular components, cell membrane, nucleus etc. Nuclear peroxidase might be involved in the structural organization of chromosome. Using aromatic amines peroxidase catalyzes redox reaction for the breakdown of $H_2O_2$.

According to Welinder (1992) peroxidases contain heam at the center. Peroxidases are classified as mitochondria, Chloroplast and bacteria class I enzymes, fungi class II enzymes and classical plant peroxidases are class III enzymes. The structure of this enzyme is well understood in peanut Van (1987), Van and Esnault (1992) and Schuller *et al.,* (1996) studied structure and function of peanut peroxidases. Plant peroxidases are glycoproteins.

Peroxidase plays vital role in lignin synthesis and auxin catabolism (Mader and Fussl 1982, Breda *et al.,* 1993). This clearly indicates role of peroxidase in plant growth and development. Van Huystee's (1987) suggested that peroxidase can inhibit cell growth. Kadioglu and Durmus (1997) reported that peroxidase activity was reduced by benzyladenine (BA a cytokinin) in cotyledons of squansh.

Gibberellic acid stimulated peroxidase under moisture stress (Vora *et al.,* 1976). In contrast to this Ram *et al.,* (1976) observed inhibition of peroxidase activity by GA, IAA and NAA, which resulted in, increase in growth of *Phaseolus* seedlings. Sengupta *et al.,* (1977) also reported similar finding with GA3, kinetin and ABA in rice. Sangeeta and Varshney (1991) also found

**Fig. 25: Effect of salicylic acid on activity of enzyme peroxidase during wheat seed germination.**

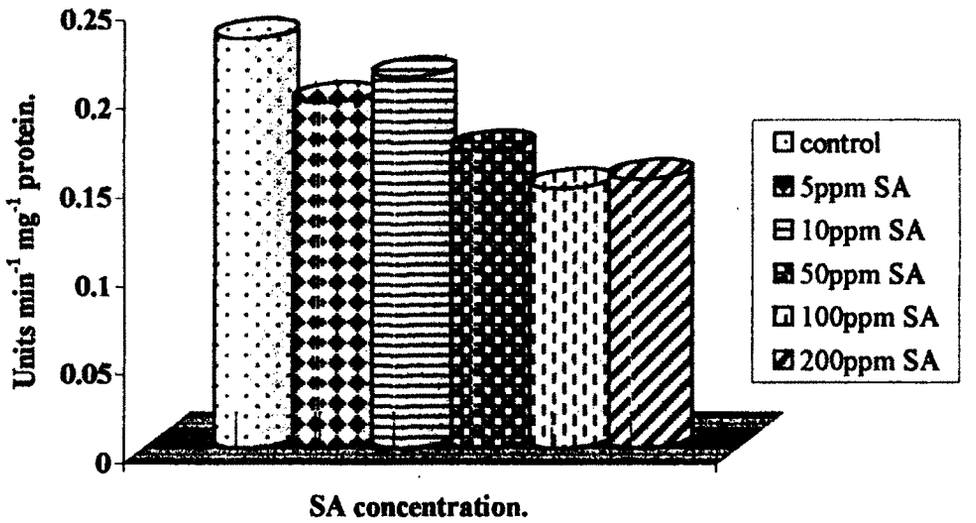
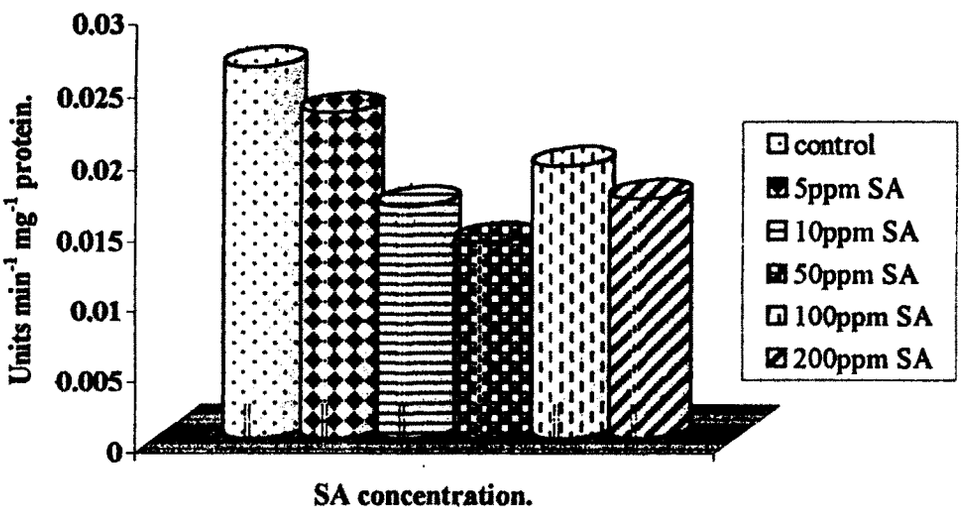



**Fig. 26: Effect of salicylic acid on activity of enzyme peroxidase during moong seed germination.**



inhibition of peroxidase by $GA_3$ treatment in *Avena sativa*. Kaul and Farooq (1992) observed inhibition of peroxidase by kinetin in *Ipomea purpurea* seedling.

Ethylene treatment enhanced peroxidase activity in senescing cucumber cotyledons. (Ables *et al.,* 1988).

Phenolic compounds also affect activity of enzyme peroxidase. Kumar *et al.,* (1981a) found increase in enzyme activity in SA treated *Impatiens balsamina* stem and leaves. Many workers have suggested that SA affects peroxidase of several plant species during defense. Jozsef *et al.,* (1997) found increase in ascorbate peroxidase activity by SA treatments. Chary and Rao (1997) found increase in peroxidase activity of intact primary leaf of bean. Quinones stimulated peroxidase activity in leaf discs of radish (Chary and Rao 1998)

Recently Mishra and Chaudhary (2001) reported decrease in peroxidase activity by SA treatment in rice. Similar to rice, we have noticed decrease in peroxidase activity by SA treatment in both the crop plants. The decrease in peroxidase may contribute to increase in intensity of oxidative stress in SA treated seedlings.

**B] PIGMENT ANALYSIS:**

**a) Chlorophylls:-**

The influence of salicylic acid on photosynthetic pigments in leaves of seedlings of wheat and moong recorded in Figures 27-34. It is clear from the figures that in wheat level, of Chl a and Chl b is decreased in all treatments. The decrease in the pigments is very insignificant in 10 ppm SA treatment. Since both Chl a and b are affected in similar manner, total Chlorophyll's also show a decreasing trend over the entire range. In case of moong, treatments (upto 100 ppm) have caused increase in the level of Chl a, Chl b (Figures 28, 30), while in 200ppm SA treatment the level of both pigment is decreased. While the Chlorophyll a/b ratio is slightly decreased by SA treatments in both the crops.

**Fig. 27: Effect of salicylic acid on chl a content in wheat seedlings.**

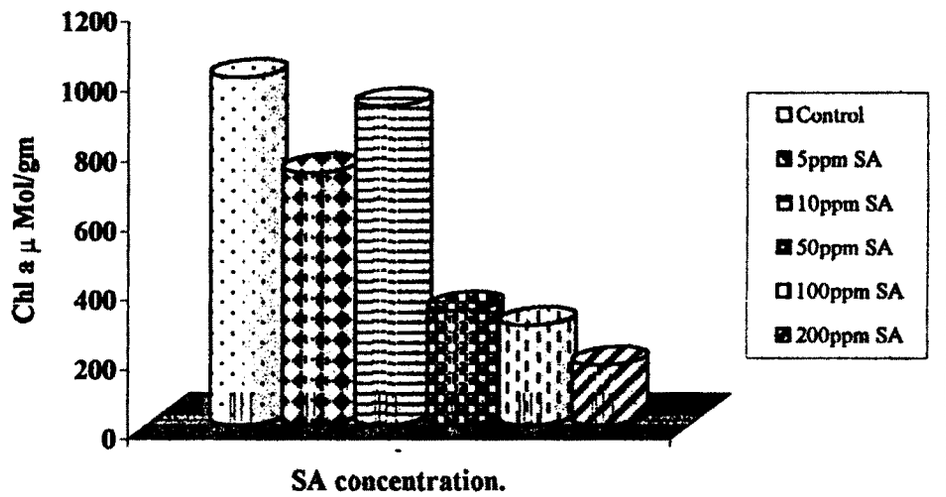



**Fig. 28: Effect of salicylic acid on chl a content in moong seedlings.**

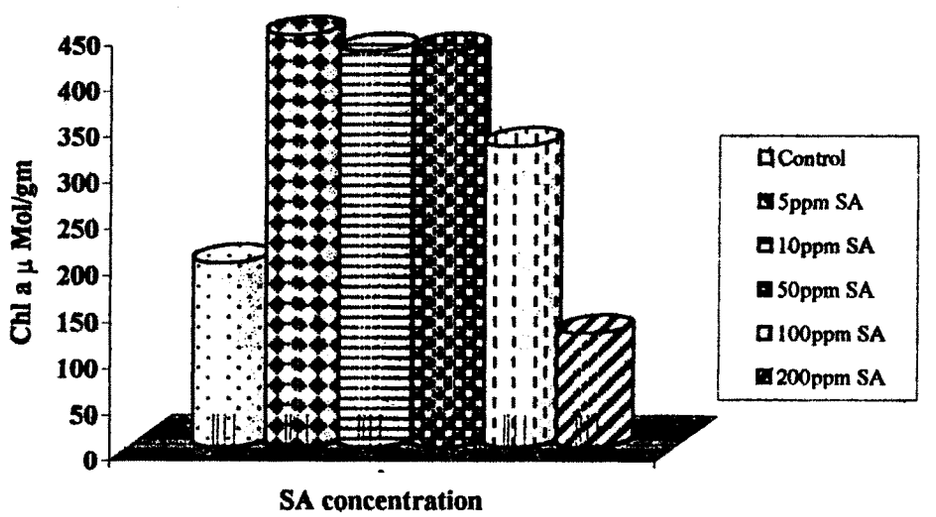


**Fig. 29: Effect of salicylic acid on chl b content in wheat seedlings.**

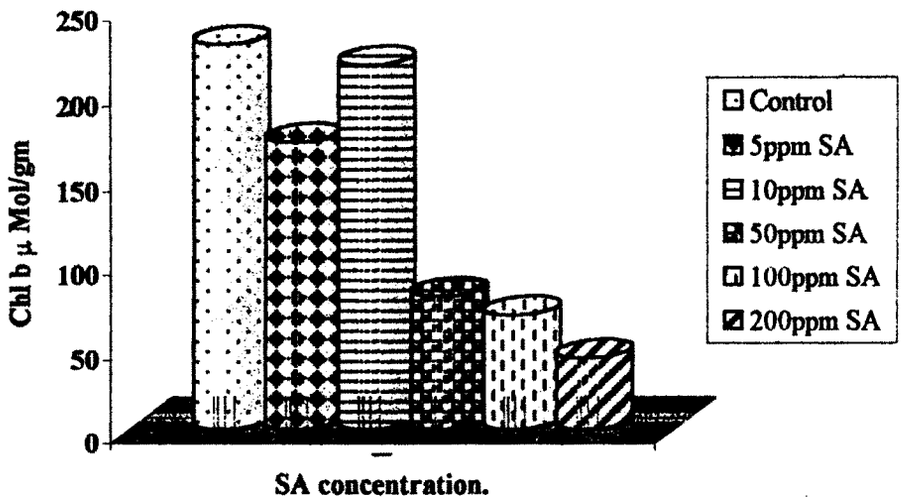



**Fig. 30: Effect of salicylic acidon chl b content in moong seedlings.**

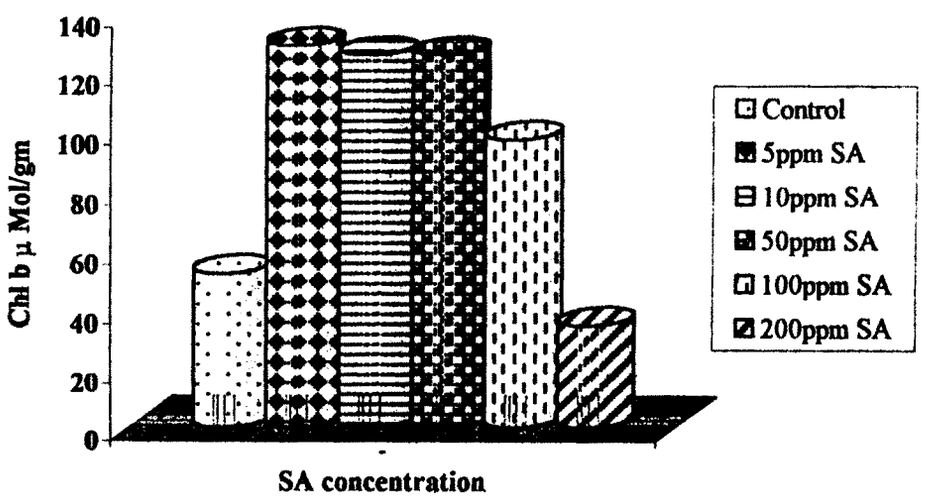


**Fig. 31: Effect of salicylic acid on chl a+b in wheat seedlings.**

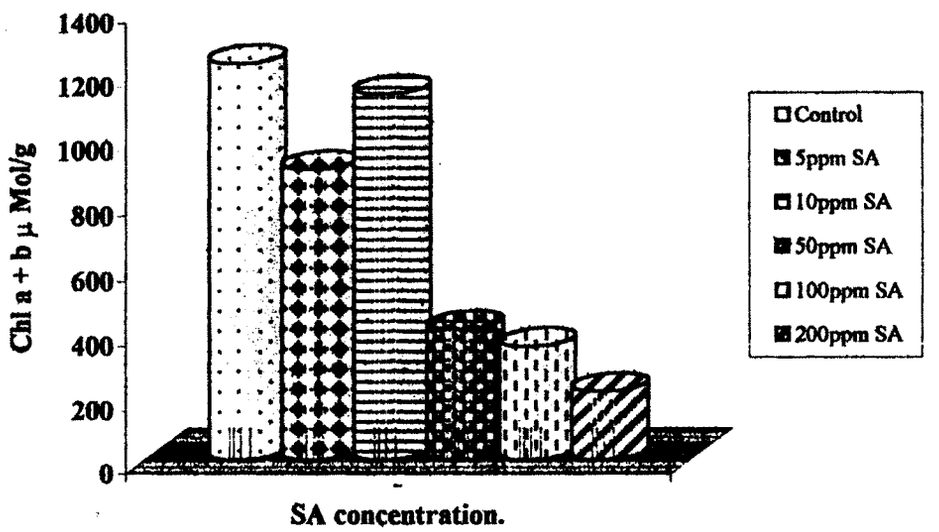



**Fig. 32: Effect ofsalicylic acid on chla+b in moong seedlings.**

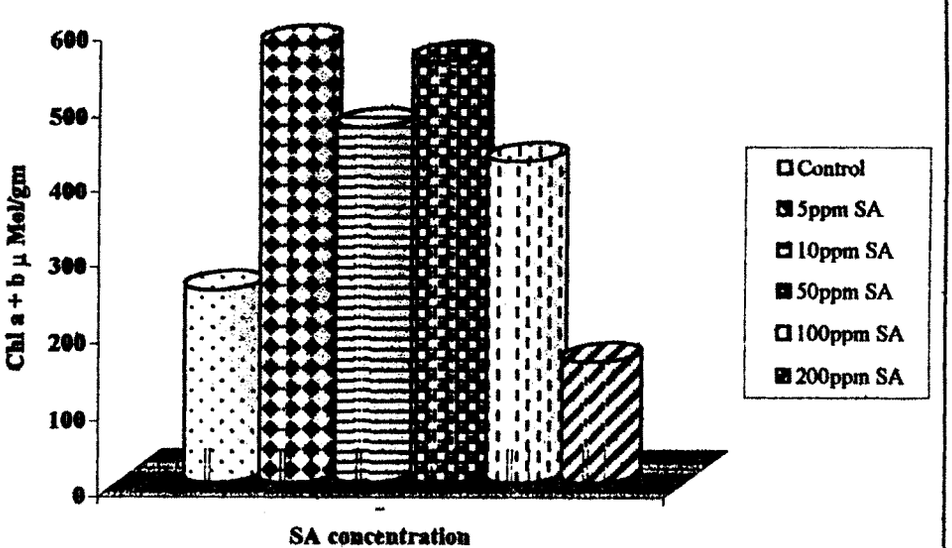


**Fig. 33: Effect of salicylic acid on chl a/b ratio in wheat seedlings.**

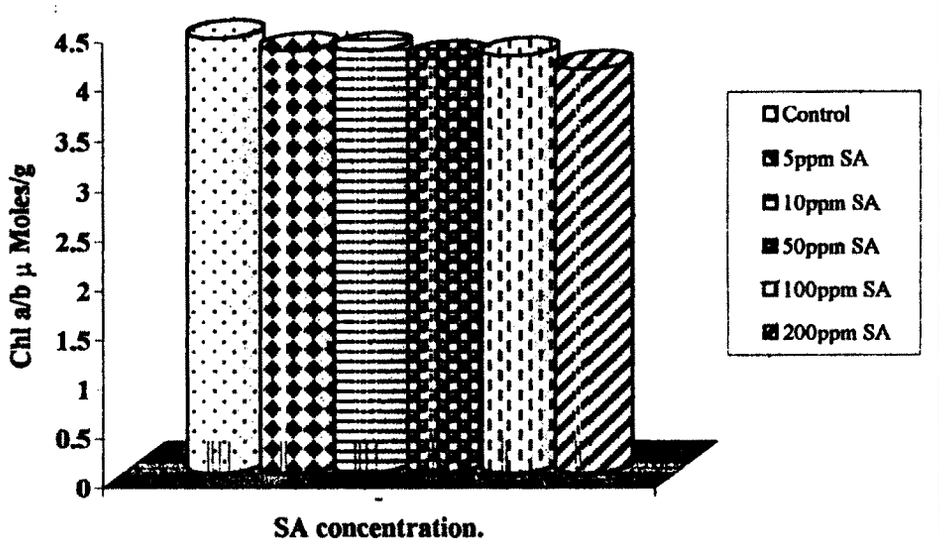
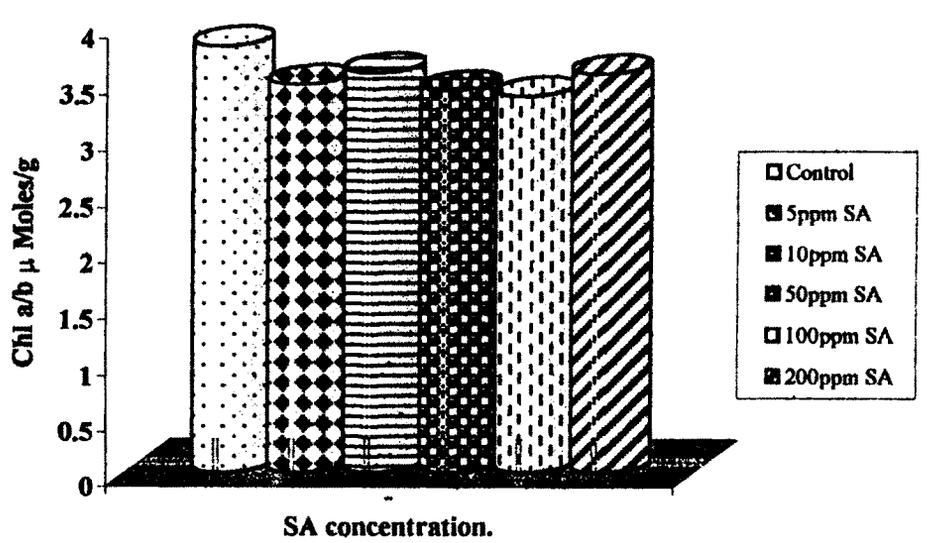



**Fig. 34: Effect of salicylic acid on chla/b ratio in moong seedlings.**



Chlorophylls are plant pigments of paramount significance as they harvest the solar energy and convert it to chemical energy. Basically Chlorophylls are the magnesium chelates of closed tetrapyrrole rings derived from protoporphyrin through a number of steps which together with iron-protoporphyrin represent the end products of porphyrin metabolism in plants. The process of photochemical charge separation is initiated by the absorption of photon by one of the 200 antenna chlorophyll molecules associated with each PSI reaction center (Golbeck 1992). Higher plants are characterized by the presence of Chl a and Chl b which are constituents of photosynthetic apparatus. Among the two Chlorophylls, Chlorophyll a plays a key role in light reaction while Chlorophyll b plays a secondary role. Since Chlorophylls play a key role in light reaction of photosynthesis, the level and state of these pigments have direct influence on the photosynthetic efficiency. The biosynthesis of Chlorophyll and the biogenesis of chloroplasts in higher plants involve a closely correlated series of events, many of which are subject to inhibition by exogenously applied chemical agents. Several observations indicate that PGRs influence the level of these pigments in the leaf tissue.

Sestak and Ullman (1960) observed decrease in Chlorophyll content but Chlorophyll a/b ration remained unchanged after GA treatment in experiment with greening of etiolated seedlings of wheat. Wolf and Haber (1960) found no effect of $GA_3$ upon the rate of Chlorophyll disappearance in darkness. They observed decrease in the Chlorophyll content of wheat when expressed on a fresh weight basis but not on per plant basis. They concluded that the effect of $GA_3$ was due to a failure of Chlorophyll synthesis to keep pace with increased growth. However Khare and Gauriprasad (1991) noted no effect of $GA_3$ on chlorophyll synthesis in radish cotyledons.

2, 4-D inhibited Chlorophyll synthesis partially in tissue cultures of *Acer* and completely in *Oxalis disper* and *Haplopapus* (Sunderland 1966) Lichtenthaler and Becker (1970) found that etiolated barley seedlings exposed to

10 ppm Abscisic acid developed only fifty percent of the normal quantity of Chlorophyll. Goswami *et al.,* (1986) observed that ABA promoted loss of Chlorophyll but they further noticed prevention of Chlorophyll loss by Benzyladenine in dark incubated dark detached sunflower leaves. Bharadwaj and Warrier (1986) noticed ABA induced decrease in chlorophyll. NAA application to black gram caused increase in chlorophyll content (Lakamma and Subbarao 1996).

Chander *et al.,* (1988) found that foliar spray of different concentrations of salicylic acid, resorcinol and tannic acid on *Vigna mungo* resulted in increase in Chlorophyll content in different parts of developing fruit. On the other hand Pancheva *et al.,* (1998) observed decreased in Chlorophyll content in barley subjected to salicylic acid treatment. Our observations also indicate a similar trend since in moong (dicot) SA treatment has brought about elevation of Chlorophyll level while in wheat (monocot) opposite trend is noticeable.

In case of wheat, the level of Chl a, Chl b and Chl a/b ratio is reduced but in case of moong the level of Chl a, Chl b and Chl a+b increased except in 200 ppm SA treatment. The Chl a/b ratio is decreased in both the species. These observations indicate that biosynthesis and accumulation of Chlorophylls in response to salicylic acid treatment differs in two species.

Thus it is clear that the Chlorophyll biosynthetic machinery in dicot (moong) and monocot (wheat) responds in a different manner to the treatment of SA.

**b) CAROTENOIDS:-**

Influence of SA treatment on carotenoid pigments in leaves of seedlings of wheat and moong is depicted in Figures 35-50. It is clear from Figures that the levels of Viloxanthin, Anthraxanthin, Lutein, and Zeaxanthin are increased with higher (50,100 and 200 ppm) doses of SA, Xanthophyll pool is also increased by higher treatments in case of wheat. The Z + A/ V + A + Z ratio (epoxidation rate)

**Fig. 35 Effect of salicylic acid on Violoxanthin content in wheat seedlings.**

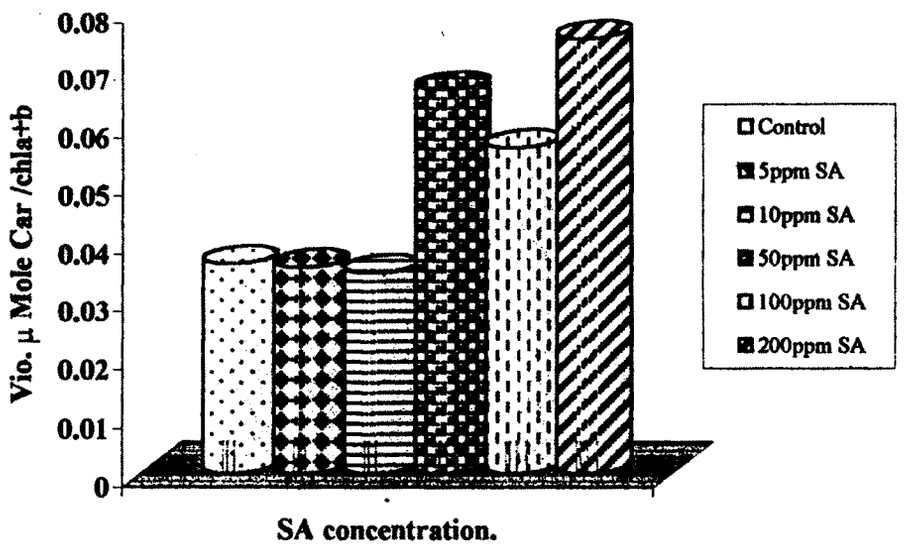



**Fig. 36: Effect of salicylic acid on Viloxanthin content in moong seedlings.**

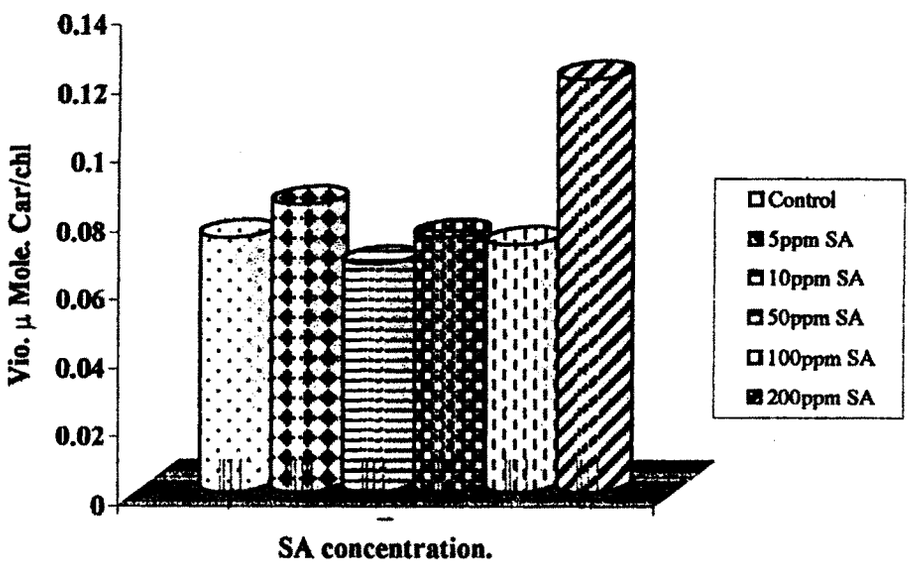


**Fig. 37: Effect ofsalicylic acid on Antheraxanthin content in wheat seedlings.**

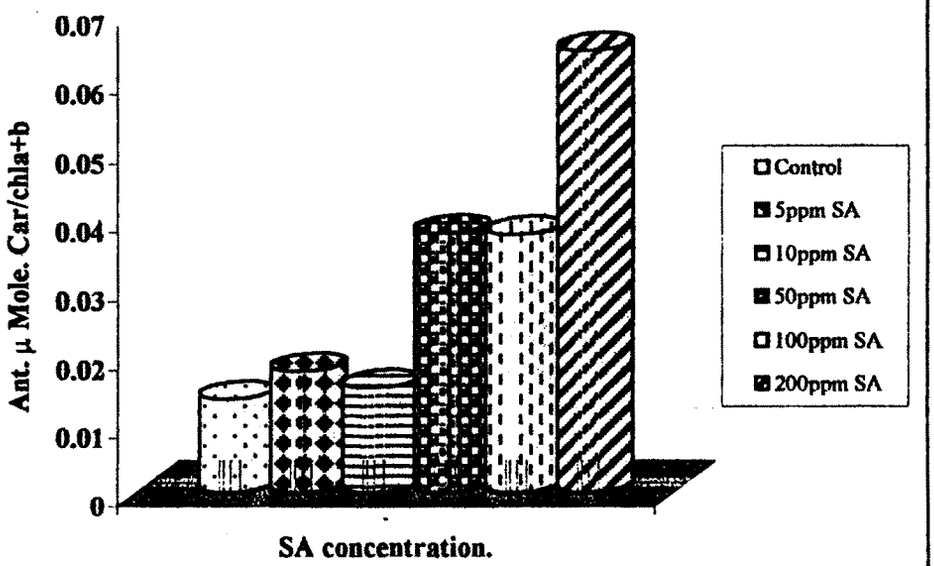



**Fig. 38: Effect of salicylic acid on Anthraxanthin in moong seedlings.**

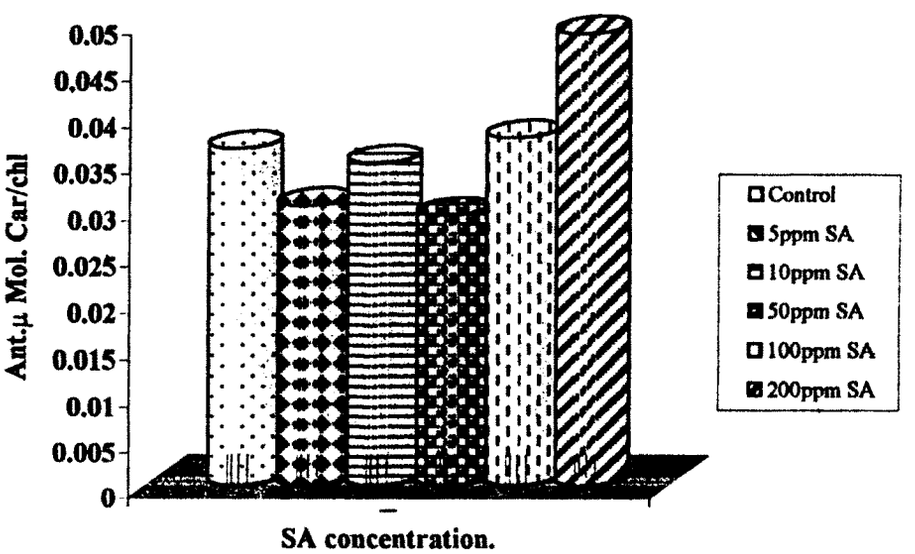


**Fig. 39: Effect of SA on Lutein content in wheat seedlings.**

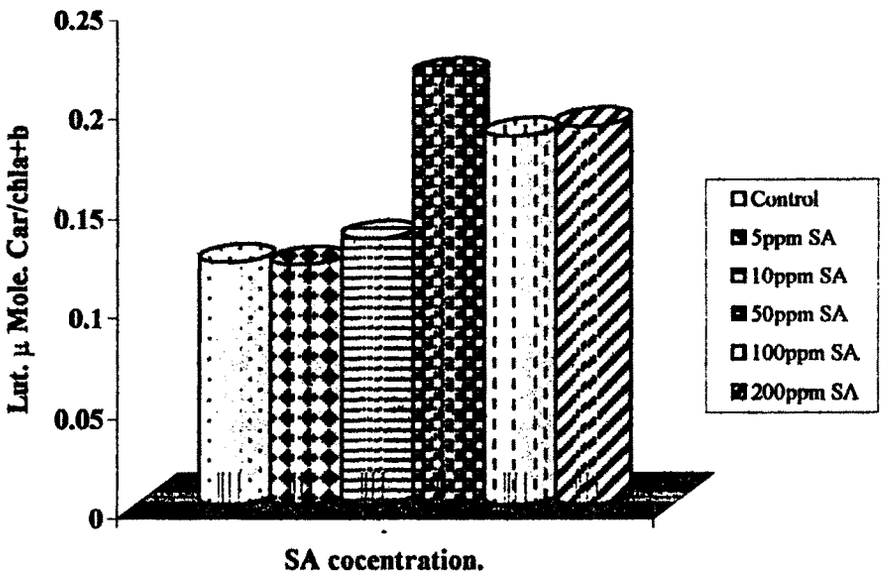



**Fig. 40: Effect of salicylic acid on Lutein content in moong seedlings.**

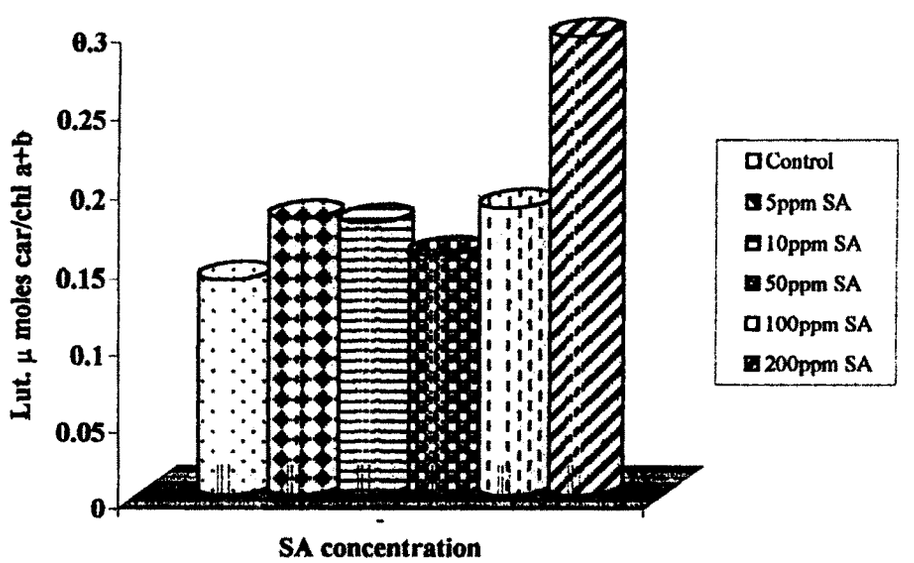


**Fig. 41: Effect of salicylic acid on Zeaxanthin content in wheat seedlings.**

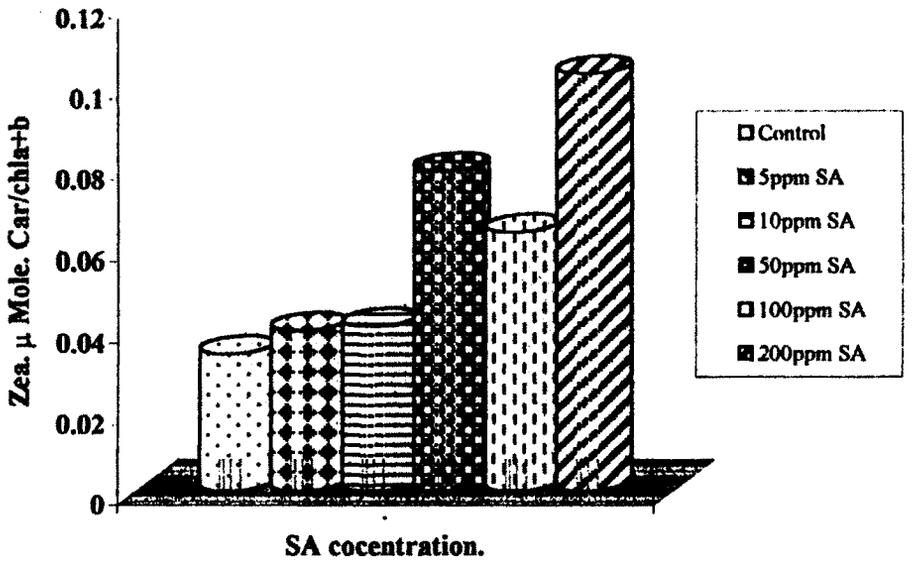



**Fig. 42: Effect of salicylic acid on Zeaxanthin content in moong seedlings.**

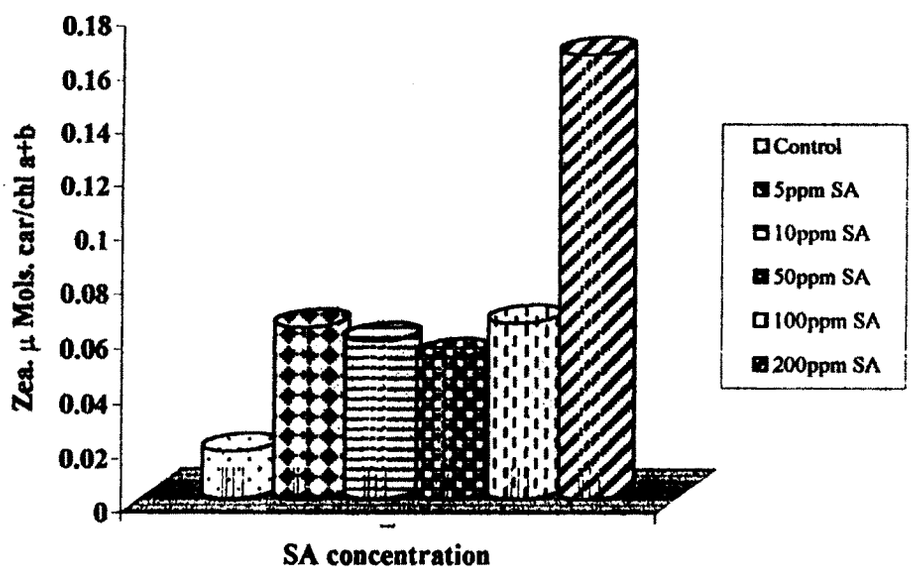


**Fig. 43: Effect of salicylic acid on β–carotene content in wheat seedlings.**

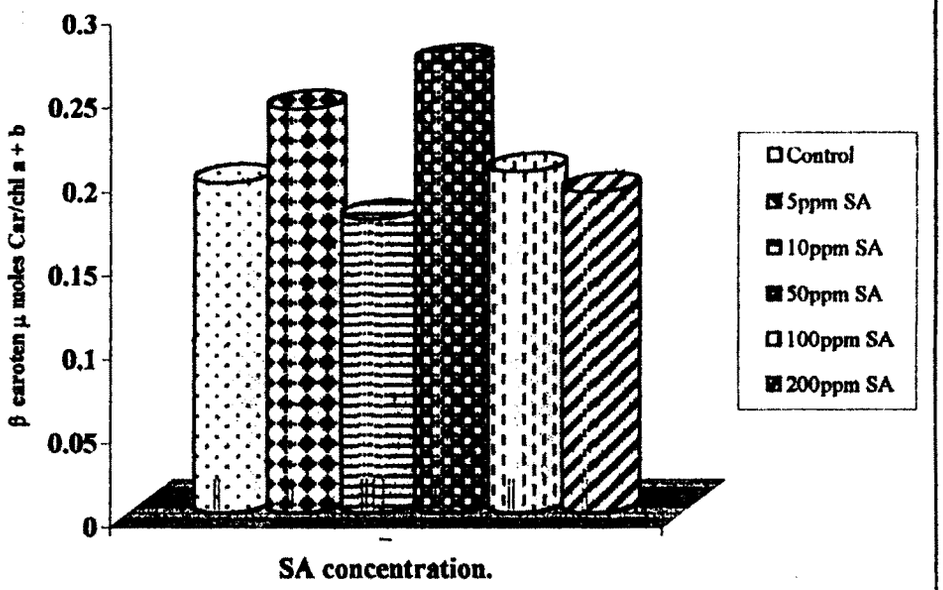



**Fig. 44: Effect of salicylic acid on β–carotene in moong seedlings.**

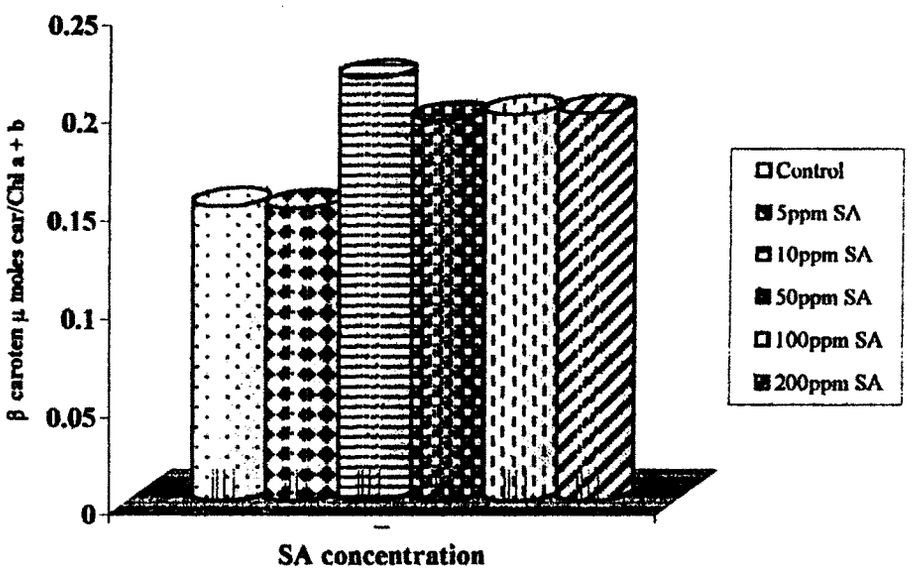


**Fig.45: Effect of salicylic acid on Total Carotenoids car/chl in wheat seedlings.**

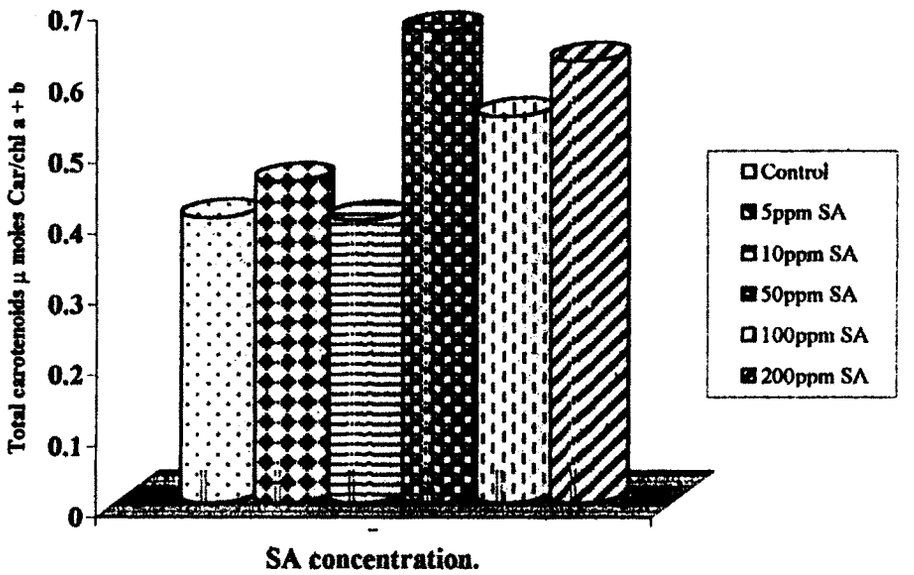



**Fig. 46: Effect of salicylic acid on total Carotenoid in moong seedlings.**

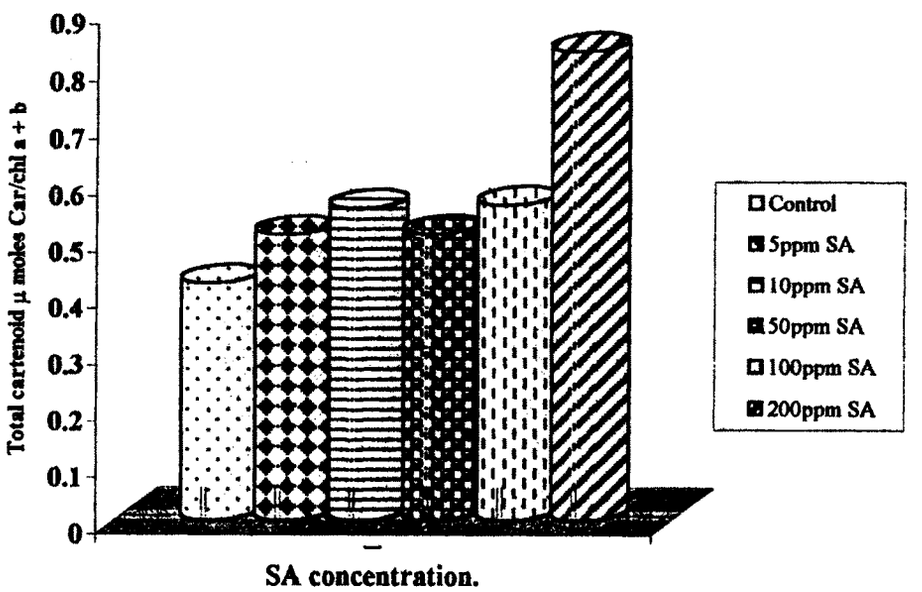


**Fig. 47: Effect of salicylic acid on xanthophyll pool in wheat seedlings.**

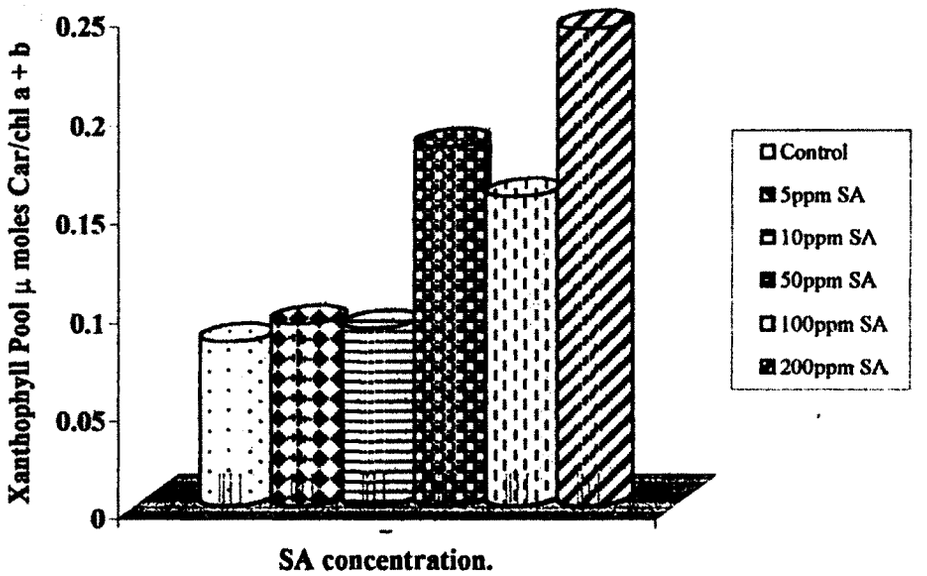



**Fig. 48: Effect of salicylic acid on Xanthophyll pool in moong seedlings.**

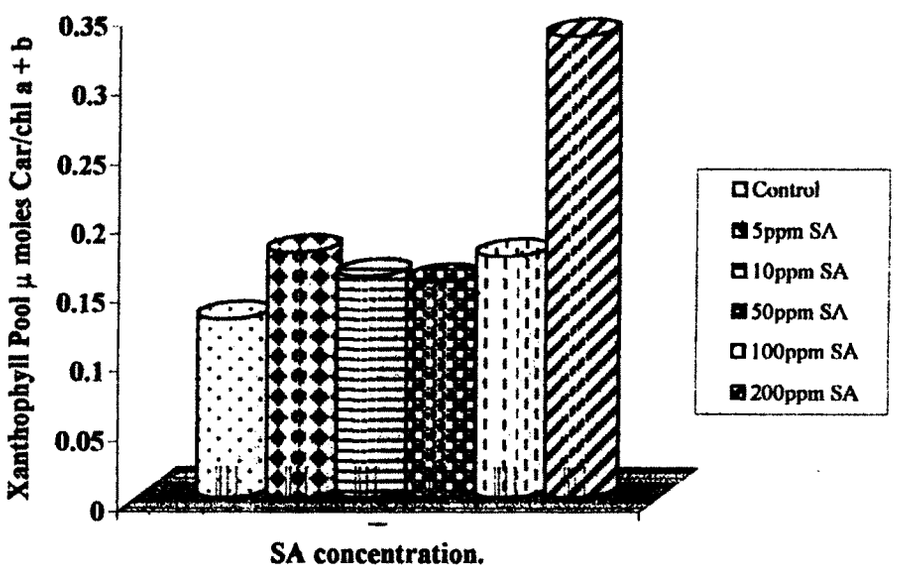


**Fig. 49: Efrfect of salicylic acid on Z+A/V+A+Z in wheat seedlings.**

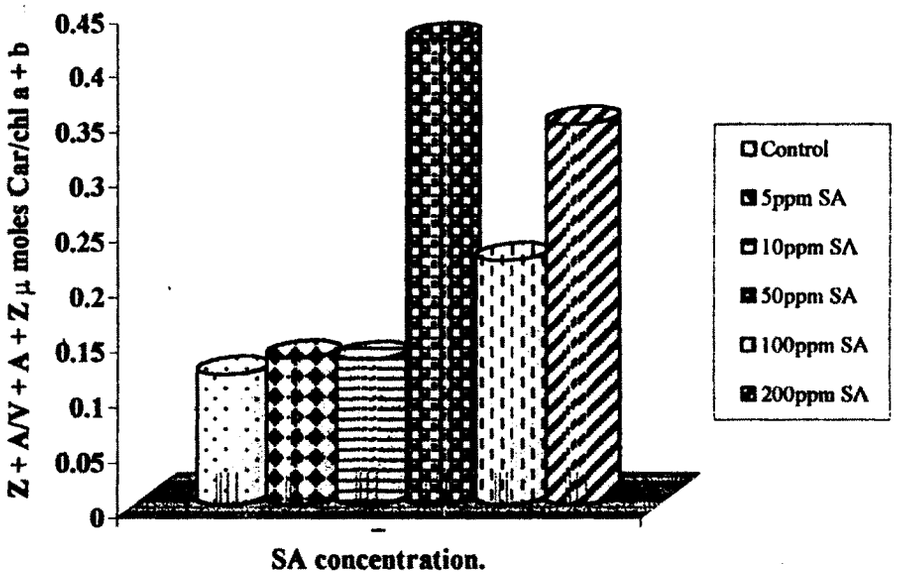
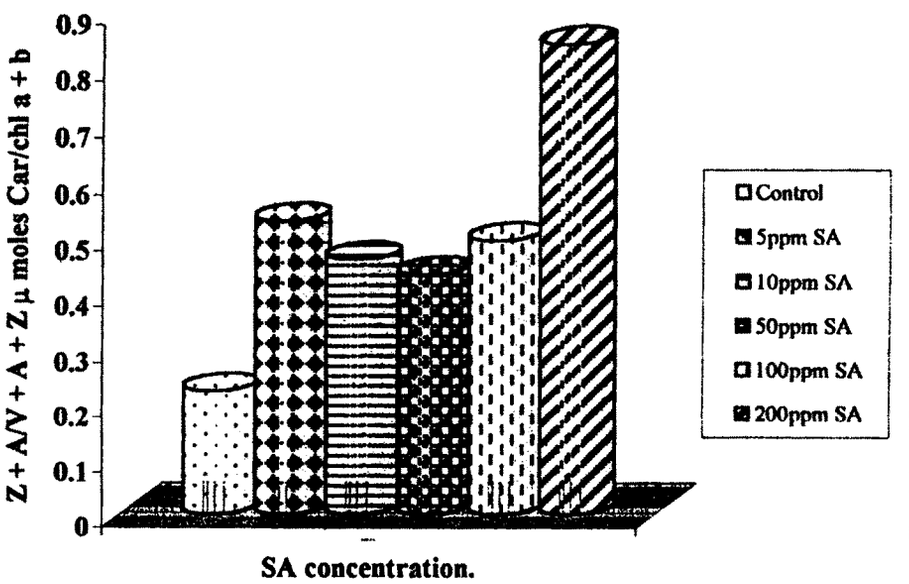



**Fig. 50: Effect of salicylic acid on Z+A/V+A+Z in moomg seedlings.**



is also increased with higher doses of SA. Level of β-carotene is slightly decreased in 10 ppm SA treatment surprisingly. The total carotenoid content is increased by all treatment except in 10 ppm SA treatment. In case of moong content of Viloxanthin, Anthraxanthin, Lutein, Zeaxanthin and Xanthophyll pool were markedly increased in 200ppm SA treatment. β-carotene and total carotenoid content increased with the increasing concentration of SA. The epoxidation rate (Z+A/V+Z+A) is also increased in all treatments.

Carotenoid pigments are ubiquitous in photosynthetic organism. In cynobacteria and plastids of higher plants carotenoids fulfill critical structural and functional roles in photosynthesis. Carotenoids provide photoprotective functions within the multisubunit photosystemes (PS) complexes. They help to maintain the three dimensional integrity of the photosynthetic complexes and their architecture within the chloroplast thylakoid membrane. They constitute accessory pigments that capture channel energy from light to Chlorophyll for the oxidation of water in the hill reaction of photosynthesis.

PS I and PS II are multisubunit complexes and structural roles for carotenoids within these complexes have been documented at both the subunit and whole-complex levels. The arrangement of the individual components in the supramolecular PS structure is designed to facilate a flexible compromise between maximal photosynthetic efficiency and minimal photooxidative damage (Horton *et al.,* 1996). It is proposed that carotenoids be involved in assembly and functions as entire complex as well as in dynamic changes in the arrangement of the individual PS components in response to changes in light regime (Horton *et al.,* 1996).

The role of carotenoids in light harvesting and photoprotection is well understood. Carotenoids harvest light of wavelength not efficiently absorbed by Chlorophyll. This energy is immediately transferred to the Chlorophylls, $P_{680}$, in the PS II reaction center. The resulting excited state of $P_{680}$ or the Singlet State is ordinarily rapidly quenched during the oxidation of water to oxygen ($O_2$). The

plants are usually getting more light and also harvest more light and convert light energy to chemical energy. Under these conditions $P_{680}$ transfer electrons directly to $O_2$ and generate reactive oxygen species (ROS). ROS causes extensive damages to photosynthetic membrane and hence very harmful for plant. Carotenoids play vital role in protection against photooxidative damage from ROS. Carotenoids especially β-carotene can directly quench ROS as well as singlet chlorophyll (Young, 1993). Alternatively excess energy from Chlorophyll can be quenched before it reaches the reaction center by the carotenoids Zeaxanthin and Antheraxanthin which are generated by the depoxidation of Viloxanthin. The lower energy state of Zeaxanthin and Anthraxanthin allows energy transfer from Chlorophylls to these carotenoids, this energy is dissipated as heat (Demmig Adams and Adams 1996). These reactions which lead to accumulation of Zeaxanthin in high light known as xanthophyll cycle (Demmig Adams and Adams 1996)

The accumulation of all major carotenoids proceeds most often in parallel but with various velocities. In wheat the amount of carotenoids declined in a sequence Carotene-Lutein-Viloxanthin, Neoxanthin from young to old leaves (Parshina *et al.*, 1972) while in tobacco the sequence was Lutein-β-caroten-Neoxanthin-Viloxanthin (De Jang and Woodlid 1979).

Application of various chemicals to plant causes alterations in carotenoid formation leading to photodestruction of Chlorophyll (Anderson and Robertson 1960). In experiments with the greening of etiolated seedlings of wheat and maize, Sestak and Ulman (1960) noted decrease in carotenoid content after GA treatment. Sunderland (1966) noted partial inhibition of carotenoid synthesis by 2, 4-D in tissue cultures of *Oxialis dispar* and *Haplopoppus*. They further noticed that Naphthalene acetic acid also caused 2, 4-D like effects. The total quantity of xanthophylls (Lutein, Zeaxanthin, Viloxanthin, Neoxanthin) were not affected by GA treatment. ABA treatment to the excised leaf fragments of etiolated maize results in inhibition of carotenoid synthesis by thirty percent (Mercer and Pughe

1969). Bharadwaj and Warrier (1986) found decrease in carotenoid level by ABA treatment in excised cucumber cotyledon greening system. Raghava and Raghava (1989) noticed increase in carotenoid content of tomato fruits in response to IAA, $GA_3$, Kinetin, Maleic hydrazide and CCC treatments.

Our observations clearly indicate that the level of carotenoid content is increased due to salicylic acid. In case of wheat Vio, Ant, Lue and Zea, level are increased by 50, 100 and 200 ppm SA treatment. But in case of moong the level of Vio, Ant increased only in 200 ppm SA treatment while level of Leu and Zea increased in all the treatments. The level of β-carotene is increased in all concentration except 10 ppm in case of wheat while in moong β-carotene level is increased in all treatments. Xanthophyll pool is increased in all treatments in both the crop plants when compared to control. Total carotenoid content also increased due to SA treatment in both the species. These observations indicate that pattern of carotenoid biosynthesis and accumulation in the two species is more or less similar. The ratio Z+A/V+A+Z is epoxidation rate. The epoxidation rate is also increased by all SA treatments in both wheat and moong. These observations suggest salicylic acid treatment create stress condition to the plants. Increase in Zea content is also signal for stress condition.