I REVIEW OF LITERATURE

A. Introduction :

The interactions between exogenous chemicals and the leaves of agricultural crops provide a major route by which plant development and the harvesting of food and fibre yield can be influenced. Such chemicals are growth hormones, long chain fatty alcohols and antitranspirants. Among these, use of antitranspirants challenge plant physiologists to answer the questions such as 1. What will be the effect on plant of the cessation of the transpiration ? 2. What is most appropriate manner of reducing transpiration ? 3. What physiological side effects will result from different types of antitranspirants, applied to different plant ecotypes under varing ecological conditions? 4. Are they affect transpiration stream of plant or govern the metabolic functions by acquiring metabolic site of the plant?

In the present state of research, the main practical problem is the development of improved antitranspirants. Longer retaintion and greater specificity are required for antitranspirants of the stomata closing type, and greater selectivity to gases and vapours for the film forming type.

B. Different Antitranspirants :

Despite of the long time interest in an antitranspirants, dating back to Theophrastus in 300 B.C., no general review of this subject seems to have been attempted (Gale and Hagan 1966). However, looking to the importance of antitranspirants many laboratories have been engaged in studying effect of antitranspirants on plants. The chemicals which have gained importance as an antitranspirants are ß-napthoxyacetic acid (Ferri and Lex 1948), 8-hydroxy quinoline sulphate (Zelitch 1961), Phenyl mercuric acetate (Zelitch and Waggoner 1962), Atrazine (Wills and Davis 1962), Alkaneyl succinic acid (Zelitch 1964), Decenyl succinic acid (Kozlowski and Clausen 1967), Abscisic acid (Milborrow 1969, Jones and Mansfield, 1970), Morphactin (Das et al. 1977).

The reduction in transpiration rate was also achieved by using material like higher alcohols, waxes, silicon, and plastics such as polyethelene, vinyl acereylate and similar polymer (Crafts 1968). In addition, synthetic long chain fatty alcohols such as HICO-110-R and ACITOLTA-1618 have been introduced in the market as a Water Evaporation Retarding Chemicals (WERCs). The work carried out by using above mentioned antitranspirants has been reviewed.

C. Overview of work done :

As early in 1948 Ferri and Lex showed that an aqueous solution of ß-napthoxyacetic acid applied to soil closes the stomates of nasturtium leaves. Zelitch (1961) found that 8-hydroxy quinoline

sulphate prevents closed stomates of tobacco leaves from opening under conditions, favourable to opening. Stodderd and Miller (1962) tested the effects of this chemicals on strawberry plants and reported that it closes stomates and reduces water loss, enabling the plants to withstand prolonged drought. Smith and Buchholtz (1962, 1964) and Wills and Davis (1962), observed reduced transpiratiion of plants treated with 2-chloro-4-ethylamino-6-isopropylamino-1,3,5,-triazine (Atrazine), and they attributed this to closure of stomates. Freney (1965) indicated superior growth of plants and increased mineral uptake in the presence of low amount of triazine herbicides, while, Pallas and Bertrand (1966) found no effect of atrazine on corn in their field trials.

Effect of phenyl mercuric acetate (PMA) has been studied on transpiration of tobacco and sunflower and on stomatal closure, transpiration and photosynthesis of corn by Shimshi (1963a,b). Their study proved that PMA reduces photosynthesis less than that of transpiration Further, analysis of resistance to water movement indicated that as soil dried, an appreciable resistance to water movement develops at evaporating surfaces of mesophyll cells by increasing water tension at these surfaces upto -80 bars without wilting the plants.

Gale and Hagan (1966) have written an excellent review on antitranspirants. They discussed the cooling effects of transpiration but pointed out that in hot, arid regions, when soil moisture becomes limiting, stomates usually close during the hotest hours of day and

thus the cooling effect is lost. Further they have stated that anti-transpirant that close stomata cause the leaves to warm up. However they are of the opinion that, because of heat conduction to the atmosphere, it is doubtful if the cooling by transpiration or warming because of antitranspirants are important factor in plant growth except under the most severe conditions. Slatyer and Bierhuizen (1964) found leaf air temperature differences upto $9^{o}C$ when transpiration was completely inhibited, in cotton plants in Australia.

Gale and Hagan (1966) pointed out that diffusion of water vapour is due to two resistances, stomatal aperture resistance and boundry layer resistance, CO_2 diffusion to chloroplasts is subject to three separate resistances : the two just mentioned plus the liquid phase diffusion resistance to movement from the mesophyll walls to the chloroplasts. Using the calculations of Gaastra (1959), they concluded that under certain conditiions an increase in stomatal resistance will reduce transpiration more than photosynthesis resulting in a favourable photosynthesis/transpiration (P/T) ratio. Work of Zelitch (1961), Zelitch and Waggoner (1962), and Slatyer and Bierhuizen (1964) showed that this may be accomplished by use of certain antitranspirants e. g., Phenylmercuric acetate (PMA).

Antitranspirants have been proposed as a means of conserving water for a critical period in development of crop (Begg and Turner 1976), and hence most of the workers are engaged in studying their effect on plant under water stress condition.

Recently Raghavendra et al. (1976) have studied the characterization of abscisic acid inhibition of stomatal opening in isolated epidermal strips of Commelina benghalensis. Their study revealed that abscisic acid (ABA) inhibited the light induced opening of stomata in isolated epidermal strips of Commelina benghalensis. It did not alter stomatal closure in the dark. The ABA-induced inhibition in light was released under conditions conducive. For cyclic photophosphorylation and remarkably reserved by ATP in the presence of pyruvate cyclic photophosphorylation rate of isolated guard cell chloroplasts were significantly reduced by ABA. Based on this investigation they have proposed that the direct effect of ABA on stomatal opening was mediated in two ways : 1. By inhibition of cyclic photophosphorylation activities of guard cell chloroplasts and 2. By blocking organic acid formation in guard cell.

Further, Das et al. (1976) have studied the effect of three morphactins, chloroflurenol, flurenol and EMD 7301 W, on strips of Commelina benghalensis. Morphactins produced striking decrease in the stomatal opening in light but had no stomatal closure in darkness. Various catalysts and inhibitors of phosphorylation had no influence on the morphactin-induced stomatal closure. The stimulatory effects of ATP, Pyruvate, KCl on stomatal opening were suppressed by the morphactins. The cytokinin, benzyladenine stimulated the stomatal opening even in the presence of morphactin. The influence of morphactins on the stomatal aperture closely resembled the effects of abscisic acid. Besides, Das et al. (1977) have also studied the anti-

-transpirant activity of morphactin on cotton plants. A single foliar application of the morphactin, EMD-7301 W (Methyl-ester of chloroflurenol) at conc. of 5 and 10 mg/lit to 7 week-old cotton plants (Gossypium hirsutum L. var. Lakshmi) produced a significant decrease of size of stomatal aperture, stomatal conductance, rate of transpiration and transpiration ratio till 9 days after the chemical application. Based on the experimental evidence they have suggested that the morphactin thus seem to function as an antitranspirant and because of its prolonged action it may be suitable for protecting field crops during shorter period of drought.

Patil and De (1976) have studied influence of antitranspirants on Brassica campestris under water stressed and non stressed conditions. The results indicated that water use efficiency was increased at low soil moisture and by antitranspirant treatment. The relative water content of leaves was reduced by low soil moisture but was increased by the antitranspirants which relieved plant water stress.

Effect of antitranspirant (PMA) on tea plant has been studied by Nagarajah and Ratnasooriya (1977). They have found that a spray of PMA reduced transpiration with gradually diminishing intensity for about 20 days, reduced vegetative growth of young plants grown both under non water stressed and water stressed conditions and reduced the yield of crop of mature plant during a drought. The relationship between stomatal conductance and capacity for assimilation was also investigated in Flacca, a mutant of tomato under influence of ABA treatment (Bradford et al. 1983). They have reported that 30 μM ABA

foliar application causes reduction in stomatal conductance by increasing CO_2 pressure. Decrease in transpiration rate and stomatal closure with increase in hydraulic conductance in ABA sprayed tomato mutants was also observed by Bradford (1983).

Agarwal et al. (1986), have studied growth of Vigna unguiculata var. GWL K3B in suboptimal moisture conditions has influenced by antitranspirants. Their study on evaluation of various aspects of growth, such as seedling survival percentage, shoot growth, root growth stomatal index and chlorophyll content indicated that application of chloro cholin-chloride (CCC) and Phoston-D can help withstanding the adverse effects of suboptimal soil moisture conditions to a certain extent.

Effects of soil moisture regime and different antitranspirant sprays on rate of transpiration and dry matter production in sunflower has been studied by Mungse and Bhapkar (1984). According to them daily transpiration rate of sunflower plants decreased under low soil moisture regime and antitranspirant treatments to the extent of 24.88 and 41 to 51.2% respectively. Shrinivasa Rao (1985) has studied the effects of antitranspirants on leaf water status, stomatal resistance and yield in tomato plant. Their study revealed that stomatal diffusive resistance significantly increased in the phenyl mercuric acetate treated plants compared with control plants. Further, they have reported that a single spray of antitranspirant can improve the plant water status and yield. Effects of antitranspirants on yield of sugarcane has also been studied by Girase et al. (1985) and reported

increase in the yield. Reduction of severe wilting by foliar application of antitranspirants in Hydragea macrophylla was observed (Mcdaniel 1985). According to him increase in leaf diffusive resistance and reduced transpiration rate may be responsible for reduction of severe wilting of Hydragea. Influence of antitranspirant and a hydrogel on net photosynthesis and water loss of Cineraria during water stress has been studied by Tu et al. (1985). Their data suggest that water loss was lowest for plants treated with antitranspirant whereas controlled plants showed loss of water under water stress condition.

Recovery from chilling injury in coleus and cucumber due to foliar application of abscisic acid was reported by Peter et al. (1986). They are of the opinion that abscisic acid provide protection against chilling symptoms.

Effect of antitranspirant on dry matter and yield of summer groundnut has been studied by Sabale and Khuspe (1986). Their study revealed that phenyl mercuric acetate and atrazine at 5 ppm concentration can increase dry pod yield of summer grioundnut. Dry seasons sweet corn response to mulching and antitranspirants is another concern studied by Shekour et al. (1987). They have reported that the mulch and antitranspirant treatments help in increasing plant height and leaf area, dry matter production as compared to the limited irrigated control. They have concluded that antitranspirant like PMA are promising in conserving water and improving yield. However, use of kaolin spray (3%) as an antitranspirant on yield of

summer groundnut (var. Lathur 33) has no influence on pod yield (Lomte and Khuspe 1987).

Santosh Kumari and Bharti (1988) have also studied the effect of CCC and FAP on water status and yield of sunflower under simulated drought conditions. According to them, treatments with CCC and FAP increased RWC and Ψ W but decreased Ψ S and thus maintained positive turgor as compared to control plants. The treatment of CCC and FAP also helped in development of bold achenes and significantly higher seed test weight. Kadam et al. (1988) have reported increase in chlorophyll contents, active iron content and in the activity of enzymes catalase and peroxidase due to foliar application of Vipul (CH_3 $(CH_2)_{28}$ CH_2OH) in spinach.

Antitranspirant associated ABA effects on water relations and yield of transplanted bell pepper has been studied by Berkowitz and Rabin (1988). ABA application resulted in increased leaf resistance and water potential, but seedling survival and yield were inhanced due to ABA only in plots which were irrigated one day after transplanting. They concluded that antitranspirant application can reduce transplant shock and increase yield of bell pepper.

In recent year, no doubt, the control of stomatal aperture and transpiration through the exogenous application of chemicals has been receiving considerable attention. Similarly good amount of work is also being carried out on effect of antitranspirant on storage procedures (Cesar and Edgar 1983). Sutter and Hutzell (1984) have

studied use of humidity tents and antitranspirants in the acclimatization of tissue cultured plants to the green house. Their study revealed that the treatments with antitranspirants were ineffective in improving vigor and survival of plants compared to controls.

Various storage treatments were imposed on cut douglas Fir Christmas trees to measure drying by studying water potential (Jose and Proebsting 1986). The storage treatment involved the use of antitranspirant but none of the antitranspirants tested reduced moisture loss.

Prevention of deterioration of cuttings during storage with the help of antitranspirant application was studied by Paton and Schwabe (1987). According to them the pre-storage application of antitranspirant (e.g. S 600 at 20 or 40%) was deterimental. Survivibility test of seedlings after leafing out by using vapour guard and antitranspirants root dip was of much concern (Askew et al. 1985). Vapour guard used as a shoot dip and a whole plant dip had little positive effect while root dips of a starch based polymer, peat and water slurry were not beneficial.

The application of antitranspirants are not only limited for studying crop productivity under water stress condition but are also involved in salt tolerance (Malash and Flower 1984, Lotfy et al. 1987) and disease control (Kamp 1985).

Malash and Flowers (1984) have studied the effect of phenyl mercuric acetate (antitranspirant) on salt tolerance in wheat. Their

study indicated that PMA at 50 μM reduces net photosynthesis and transpiratioin but the reductioin was greater in absence rather than presence of NaCl. Furthermore PMA lowers down shoot Na^+ contents and promote the selectivity for K^+ over Na^+ under salt conditions. From this they have concluded that any beneficial effect of PMA are the consequences of improved water relations, lowered ion content and increased leaf area for photosynthesis.

The study of Lofty et al. (1987) pertaining to effect of salinity and vapor guard on cotton seedlings revealed that application of vapor guard encouraged, to some extent the growth of cotton var. Giza-4 while reverse effect was noticed in Ashmouni cultivar. Use of antitranspirant favoured nutrient uptake even under saline condition in both the cotton varieties Giza-4 and Ashmouni.

A polymer based antitranspirant was compared to a fungicide Acti-Dione PM for control of Erysiphae cichoracearum or Zinnia elegans (Kamp 1985). He reported that plants treated with antitranspirant had a significant increase in height, fresh and dry weight and length of flowering period. In addition, the antitranspirant treated plants had a significantly reduced powdery mildew.

D. Scope of the Present Investigation :

Co-ordinated efforts of plant breeders, plant physiologists and agronomists have changed very face of food grain production of the world and today relative to other crops, the production of cereals has increased many fold to cope up world's population demand. In

this respect rice, wheat, jowar are not exceptions. Same is the situation for oil seed crops such as groundnut and sunflower. The high yielding varieties of today are mainly pooled for physiological characters, such as high rate of photosynthesis, high nitrogen assimilating ability and short duration. High rate of photosynthesis again is linked with the leaf characteristics. The only constraint that, in the tropical country like India being faced is water availability. The high yielding varieties can only give better yield if adequate water is available. Genetic breeding for drought tolerance does not promise any hope for the experiences tell that high yilding and drought tolerance do not go together. The answer to this question can only be given by facilitating these high yielding varieties to conserve water, perform better under short duration drought conditions, such as unanticipated disappea rance of monsoon during crucial period of growth. Thus, water stress caused due to unpredictable rains has become an endemic problem in India more so particularly in Maharashtra. The productivity of rainfed crops, such as jowar and groundnut which are wide under cultivation, is going down very fast. One cannot afford to sustain the productivity loss if it is severe. It is, therefore, necessary to evolve alternate method effectively to handle such problem.

The large scale availability of industrially produced agrochmicals has helped to revolutionize agricultural practice by making it less labour intensive and by increasing yield potentials. In the recent years several Water Evaporation Retardent Chemicals (WRCS), which can be used as an antitranspirants are available in the market.

One of such effective chemicals is introduced by HICO Limited, Bombay. It is a combination of ethylene oxide and long chain fatty alcohols (C_{12}-C_{18}) and named as HICO-110R. Since it is an synthetic product and proved as an effective water evaporation retardent chemical as well as growth promoter (Baraskar and Tilak, 1968 personal discussion), has prompted us to study its effect on crop plants. The pilot experiment conducted in our Department regarding its effect on stomatal regulation of crop plants has revealed that it also act as an antitranspirant (unpublished) when given in the form of foliar application.

However, no scientific explanation hetherto could be given as to how such chemicals help in withstanding water stress due to lack of sustained physiological invesdtigation. It is thereofre, proposed to carry out the experiment by using HICO-110R on sorghum (M-35-1) and groundnut (JL-24) under the condition of water stress.

II MATERIALS AND METHODS

A About the chemical used in the present investigation

The concept of water conservation in lakes employing water evaporation retardant chemical films has gained prominence in the past few years. This technique has been exhaustively studied by the National Chemical Laboratory, Pune, from the technical and feasibility view point (Water Evaporation Control, NCL Monograph Series 2, July 1985). Simultaneously the Japanes workers extended this idea for increasing ambient water temperature of rice seedling plots. This resulted in enhanced rice yield.

Sapre (1986) has attempted to synthesize a series of water evaporation retardant chemical (WERC) with the view to use them in agriculture. The finished product developed by Sapre (1986) has been brought into market by HICO Products Limited, Bombay under the name HICO-110R.

HICO-110R is a composition obtained by the hydroxyethylation of fatty alcohols, containing chain length C_{12} to C_{28} carbon atoms, by interaction with ethylene oxide. The reaction products were then emulsified with water to give 35 per cent emulsions.

$$\text{Fatty alcohol } C_{12} \text{ to } C_{28} + \text{ethylene oxide} \xrightarrow{\text{hydroxyethylation}} \text{HICO 110R}$$

The pilot experiments conducted with HICO-110R with the view to use them in agriculture revealed their potentiality as a growth regulator as well as an antitranspirant. Hence this chemical (HICO-110R) has been selected to study its effect on crop plants in the present investigation.

B. Procurement of seeds :

The seeds of Sorghum M-35-1 and groundnut JL-24 were obtained from Agricultural College Kolhapur. They were surface sterilized with 0.1% $HgCl_2$ for 1 min. and then were washed repeatedly with distilled water and dried at room temperature for over night. They were sown in an earthen pot containing 3 part soil and 1 part farm yard manure. After stabilizing the seedlings upto one month, the foliar application of HICO-110-R (1 ml/lit distilled water) was given to run-off point with the help of air pneumatic spray pump. The control plants were sprayed with equal amount of distilled water. After that 4 groups were made : First group: control, Second group: sprayed control, Third group: stressed and Fourth group: sprayed and stressed. The stress was imposed by withhelding water for 8 days.

C. Preparation of pots and nature of soil :

1. Preparation of pots :

The pot size and nature of soil in an earthen pot used for water stressed experiment was as follows.

Pot size	= 38 x 38 cm
Drain out hole	= 1.5 cm in diameter
Soil per pot	= 20 Kg (3:1, soil: FYM)
Plants per pot	= Groundnut: 3, Sorghum: 2

2. Soil condition :

Water at field capacity = 73.70%
Hygroscopic water = 6.65%
Capillary water = 67.05%

3. Field capacity :

Water at field capacity, hygroscopic water and capillary water that the soil sample is capable of holding were determined by the method described by Troeh and Palmar (1970), and employing the formulae.

$$\% \text{ water at field capacity} = \frac{\text{Wet weight - Oven-dry weight}}{\text{Oven-dry weight}} \times 100$$

$$\% \text{ hygroscopic water} = \frac{\text{air-dry weight - Oven-dry weight}}{\text{Oven-dry weight}} \times 100$$

$$\% \text{ capillary water} = \frac{\text{Weight at field capacity-air-dry weight}}{\text{Oven-dry weight}} \times 100$$

D. Stomatal behaviour :

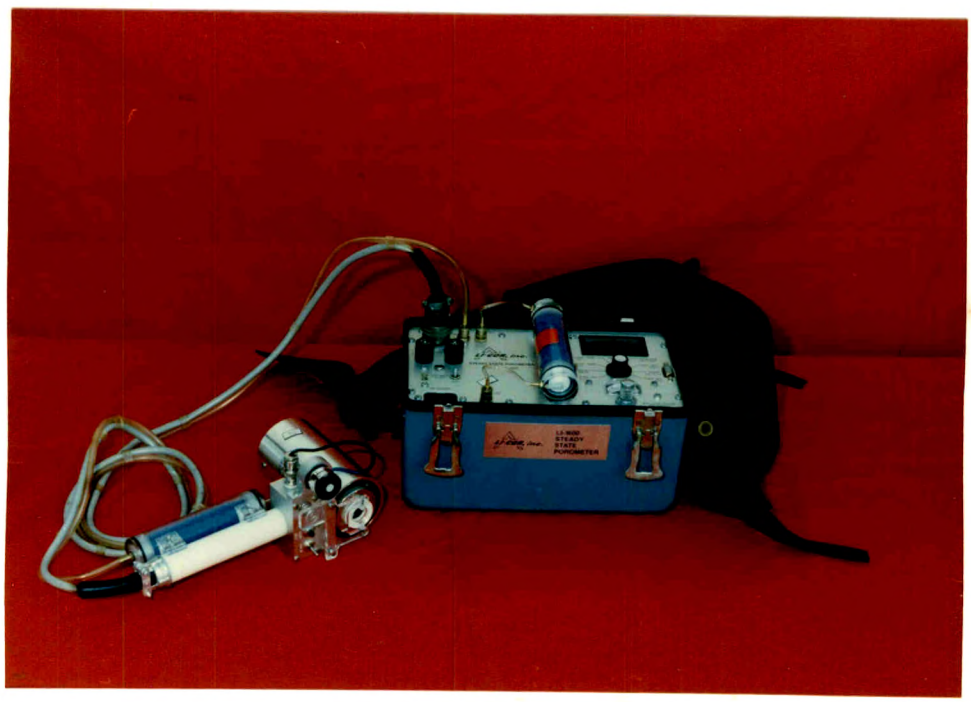
Leaf diffusive resistance and transpiration rate were determined by using steady state porometer (LI-1600, LICOR U.S.A.). The diffusive resistance for CO_2 was calculated using the formula suggested by Jarvis (1971).

$$\frac{1}{R} \text{ leaf} = \frac{1}{R} \text{ upper} + \frac{1}{R} \text{ lower}$$

$$R_L \; CO_2 = 1.6 \; R_L \; H_2O$$

PLATE - 1

Steady State Porometer used to study the stomatal regulation


LS-900
STEADY
STATE
POROMETER

E. Relative Water Content (R.W.C.) :

One g leaf discs (5 mm in diameter) were taken from different plant groups mentioned earlier. The leaf discs were kept in a conical flask containing water. After three hours, when the leaf discs attained full turgid level, weight was taken and then leaf discs were kept in an oven at 60°C. The leaf discs were allowed to dry completely and then the dry weight was noted. The per cent relative water content was estimated by using the formula

$$R.W.C.(\%) = \frac{\text{Fresh weight - Dry weight}}{\text{Weight of full turgid level-Dry weight}} \times 100$$

F. Osmotic potential of cell sap (O.P.) :

Osmotic potential of cell sap was determined by the method suggested by Janardhan et al. (1975). 1 g of fresh plant material was crushed in 10 ml of distilled water using mortar and pestle and squized through 4 layered muslin cloth. The volume of filtrate was adjusted to 25 ml with distilled water and the electrical conductance was measured on conductivity meter (ELICO Model PE-133).

Simultaneously 1 g fresh plant material was kept in an oven at 60°C to determine moisture content. Dilution factor (d.f.) was calculated with respect to moisture content of plant and the total volume of extract. Osmotic potential of cell sap was then calculated by substituting the values in the formula given below :

$$O.P. = \frac{0.36 \times E.C. \times d.f.}{0.987}$$

where,

0.36 is a constant

E.C. = Electrical conductance in mMhos/cm

d. f. = Dilution factor

0.987 = is the factor used to convert atmospheric pressure to bar

Leaf moisture, soil moisture, leaf area, and plant height was measured by routine method. Yield parameters like biomass, grain weight and earhead weight were also measured.

G. Organic constituents :

1. Total chlorophylls :

Chlorophylls were estimated following the method suggested by Arnon (1949). 0.5 g fresh material was crushed in a mortar with pestle and extracted in 80% chilled acetone containing 4 ml liq. NH_3 per litre in a dark and cold room. A pinch of $MgCO_3$ was added during crushing. This extract was filtered through Buchner's funnel using Whatman No.1 filter paper. The volume of filtrate was adjusted to 50 ml with 80% acetone. The extract was then transferred to a conical flask. This flask was covered with black paper to retain the activity of chlorophylls. The absorbance was read at 645 and 663 nm on double beam spectrophotometer (Shimadzu).

Chlorophylls (mg/100 g fresh weight) were calculated using the formulae :

Chlorophylls 'a' $= 12.7 \times A_{663} - 2.69 \times A_{645} = X$

Chlorophylls 'b' $= 22.9 \times A_{645} - 4.68 \times A_{663} = Y$

Total chlorophyls (a+b) $= 8.02 \times A_{663} + 20.20 \times A_{645} = Z$

$$\text{Total chlorophylls /100 g} = \frac{X/Y/Z \times \text{Volume of extract}}{1000 \times \text{weight of plant material (g)}} \times 100$$

2. Chlorophyll stability index :

To study the chlorophyll stability index, chlorophyll extract was prepared from 0.5 g fresh plant material as well as from 0.5 g fresh plant material kept in an oven at 60°C for 1 hour using 80% acetone by the method described above. The chlorophyll stability index was calculated by the formula given below.

$$\text{Chlorophyll stability index} = \frac{\text{Total chlorophyll content of fresh sample}}{\text{Total chlorophyll content of heated sample}}$$

3. Polyphenols :

Polyphenols were estimated by the method of Folin and Dennis (1915). 0.5 g dried plant material was crushed in a mortar with pestle and was extracted in 80% acetone. This extract was filtered through Buchner's funnel using Whatman No.1 filter paper. The residue on the filter paper was washed several times with 80% acetone and the final volume of extract was adjusted to 50 ml with 80% acetone.

2 ml of plant extract was taken in Nessler's tube along with the series of standards (Standard tannic acid 0.1 mg/ml) to which 10 ml of 20% Na_2CO_3 was added. The volume was adjusted to 35 ml with distilled water. Then 2 ml of Folin Dennis reagent (Dissolve 100 g sodium tungstate and 20 g phosphomolybdic acid in 800 ml distilled water. Add 50 ml 80% phosphoric acid. Reflux for 2 hours using water condenser. Adjust the final volume to 1 lit with distilled water) was added to each test tube and the final volume was adjusted to 50 ml with distilled water. After about 20-30 min. absorbance was read at 660 nm using reaction blank. Polyphenols were claculated from standard curve of tannic acid and were expressed in g/100 g dry tissue.

4. Estimation of proline :

Proline content was estimated by the method of Bates et al. (1973). 0.5 g dried plant material was homogenised in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate was filtered through Whatman No.1 filter paper. 2 ml of filtrate was reacted with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 h at 100°C in water bath, and the tubes were transferred to ice bath immediately. After 15 min. the reaction mixture was extracted with 4 ml toluene by shaking vigorously with a test tube stirrer for 20 sec. The red coloured toluene phase was separated from the aqueous phase with the help of small separating funnel and warmed to room temperature and the absorbance was measured at 520 nm

using toluene as a blank on Shimadzu spectrophotometer. The proline concentration was determined from a standard curve and calculated on a dry weight basis.

a) Preparation of acid-ninhydrin :

This reagent was prepared by warming 1.25 g ninhydrin (Unichem) in 30 ml glacial acetic acid (Merck) and 20 ml 6 M phosphoric acid (Glaxo) with agitation unitl dissolved. The reagent remain stable for 24 h at $4^{o}C$.

5. Estimation of nitrogen :

Nitrogen was estimated by the method of Hawk et al. (1948).

For plant digestion 0.5 g of dried plant material was taken in Kjeldahl's flask containing 10 ml of 1:1 H_2SO_4, pinch of microsalt and few glass bids. This was digested on low flame till colourless solution was obtained. Then it was cooled and transferred quantitatively to volumetric flask and the volume was made 100 ml with distilled water and filtered through Whatman No.1 filter paper. From the filtrate 2 ml of extract was taken in Nessler's tube to which a drop of 8% $KHSO_4$ was added and volume was made to 35 ml with distilled water. Then 15 ml of Nessler's reagent (freshly prepared) was added to it.

After 10-15 min the absorbance was recorded at 520 nm on double beam spectrophotometer (Shimadzu). The blank contained all the ingredients except nitrogen source.

Standard curve was obtained by using different concentrations of ammonium sulphate (0.1, 0.2, 0.3, 0.4 ml) by using same procedure of nitrogen estimation.

The values are expressed as g of nitrogen per 100 g of dry tissue.

a) Preparation of microsalt :

Microsalt was prepared by grinding unhydrous $CuSO_4$ and potassium sulphate in proportion of 1:40 i.e. 100 mg of $CuSO_4$ + 4 g of K_2SO_4.

b) Preparation of Nessler's reagent :

a) 7 g of KI + 1 g of HgI_2 dissolved in 40 ml distilled water.

b) 10 g of NaOH dissolved in 50 ml of distilled water.

Both a and b were mixed immediately before use.

c) Preparation of standard ammonium sulphate solution :

$(NH_4)_2SO_4$ was kept in an oven at 60°C for 10 h and 0.266 g of it was dissolved in water. A few drops of concentrated H_2SO_4 were added to it and the volume was made to 1 litre. This contains 0.05 mg of nitrogen/ml.

H Enzymes of nitrogen metabolism :

1. In vivo assay of nitrite reductase (E.C.1.6.6.4)

The method used for this assay was that of Guerrero (1982). 0.5 g of fresh plant material (cut pieces of leaves about 5 mm^2) was placed in a tube containing 4.5 ml of 100 mM phosphate buffer

(pH 5.0) and 0.1 mM $NaNO_2$). The tubes were evacuated with an oil pump for 2 min. They were then wrapped in aluminium foil and incubated at 30°C with gentle shaking on a rotary shaker. After 30 min the tubes were transferred to a boiling water bath for 2 min to stop the reaction and then cooled. To determine the initial nitrite concentration, one set of tubes was maintained as blank with no plant material. The final nitrite concentration was determined in 1 ml aliquots.

2. In vivo assay of nitrate reductase (E.C. 1.6.6.1) :

The method employed in assaying in vivo nitrate reductase (E.C. 1.6.6.1) activity was of Guerrero (1982). 0.5 g cut pieces of fresh leaves (about 5 mm^2 area) were added to 5 ml incubation mixture containing : 0.1 M potassium phosphate buffer, pH 7.7; 0.1 M KNO_3, and 1% (v/v) isopropanol. After flushing the mixture for about 5 min with argon the entire set was incubated in dark at 30°C. Aliquots (0.2 ml) were then removed for nitrite determination at zero time and after 60 minutes.

a) Estimation of nitrite :

One ml of 1% sulfanilamide in 1 N HCl and 1 ml of 0.02% aqueous solution of N-(1-naphthyl)-ethylene diamine dihydrochloride were added to 1 ml aliquots. The colour was allowed to develop for 20 min and the total volume was made upto 10 ml with distilled water. The absorbance was read at 540 nm and the nitrite concen-

tration was calculated from a standard curve. The activity was expressed as μg NO_2 reduced formed per g fresh weight per hour.

I. Field Trials :

Foliar application of HICO-110-R on field grown groundnut UF-70-103 (one month old) was given with Knapsac sprayer at Jaysingpur. The control was maintained by spraying well water. After three subsequent sprays (1 ml/lit water) at an interval of 15 days the ground nut leaves were examined for stomatal regulation.